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LINEAR DOSE RESPONSE CURVES IN FUNGI AND *TRADESCANTIA*

by

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Prepared for the
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LINEAR DOSE RESPONSE CURVES IN FUNGI AND *TRADESCANTIA*

A report by Dr. P. Unrau Atomic Energy of Canada Limited Chalk River Laboratories Prepared for the Advisory Committee on Radiological Protection of the Atomic Energy Control Board under the AECB Regulatory Research and Support Program

ABSTRACT

Tradescantia Clone 02 data suggests that linear non-threshold dose responses are expected to the lowest doses and dose rates of low linear energy transfer (LET) radiation. This is likely to be true for other living organisms even though Clone 02 is radiation sensitive.

It is concluded that Clone 02 is partially defective in the RAD 6 pathway for the repair of DNA interstrand cross-links (ISCL) and other loss of coding damage (LCD), based on its cross sensitivities to EMS and ionizing radiation. *Tradescantia* Clone 02 data showing linear non-threshold induction of somatic genetic events in part reflects the repair deficiency of this Clone. More DNA damage is repaired by recombinational mechanisms in Clone 02 than would occur in a wild-type strain.

Two important classes of DNA lesions are induced by ionizing radiation in DNA - double strand breaks (DSB) which are repaired by recombination mechanisms, and loss of coding information damage (LCD), which is repaired by error prone mechanisms but may also be a substrate for recombinational repair. Based on data from yeast, there are two different repair pathways which deal with these differing lesions with different somatic genetic consequences. From yeast, yield cross sections can be derived and applied to DNA damage and repair in Tradescantia.

For Clone 02, per lesion, more visible genetic events are scored than in wild-type strains. In a radiation-derived sub-clone, Clone 0106, which is more variable than Clone 02, even more events occur per lesion. This derivative clone, plus breeding experiments, indicate that Clone 02 is heterozygous, or a "carrier" for a mutant version of a gene in the *Tradescantia* RAD 6 repair pathway. Clone 02 is, therefore, much like a Fanconi's anemia carrier in a human population, while the Clone 0106 derivative is much like a homozygous Fanconi's anemia patient, with respect to its response to ionizing radiation damage.

Two anomalies in its dose response curves for "pink" loss of heterozygosity (LOH) events occur because Clone 02 repairs both DSB and LCD by recombination. Clone 02 has a linear dose response for high LET radiation. Starting from the same initial yield frequency, wild-types have a sublinear response. The sublinear response reflects a smoothly decreasing probability that "pinks" are generated as a function of increasing high LET dose for wild-type but not Clone 02. This smoothly decreasing response would be expected for LOH in "wild-type" humans. It reflects an increasing proportion of DNA damage being repaired by non-recombinational pathways and or an increasing probability of cell death with increasing dose.

Clone 02 at low doses and low dose rates of low LET radiation has a linear dose response, reflecting a 1/16 probability of a lesion leading to LOH, relative to high LET lesions. This differential is held to reflect: microdosimetric differences in energy deposition and, therefore, DNA damage by low and high LET radiations; the effects of lesion clustering after high LET on the probability of generating the end

points; and the effects of channeling lesions into repair systems which do not generate the end-points in wild-types. While no observations have been made at very low doses and dose rates in wild-types, there is no reason to suppose that the low LET linear non-threshold dose response of Clone 02 is abnormal. The importance of the LOH somatic genetic end-point is that it reflects cancer risk in humans.

The linear non-threshold low dose low LET response curves reflects either the probability that recombinational Holliday junctions are occasionally cleaved in a rare orientation to generate LOH, or the probability that low LET lesions include a small proportion of clustered events similar to high LET ionization or both.

Calculations of the Poisson probability that two or more low LET lesions will be induced in the same target suggest that dose rate effects depend upon the coincidence of DNA lesions in the same target, and that the probability of LOH depends upon lesion and repair factors. But the slope of LOH in Clone 02 and all other strains never approaches the expected slope of +2 predicted for two hit events. This suggests that only particular combinations of coincident LCD and DSB lesions lead, on repair, to LOH.

All the evidence suggests that DNA damage induction is linear to the lowest doses. The evidence suggests that even at the lowest doses and dose rates of low LET radiation, there is a constant probability of LOH arising from repair. The effects on cancer risk of this LOH will depend upon the genotype of the individual, and further selective mechanisms which may ameliorate or intensify the effects of the original LOH event.

Radiation LOH mechanisms and organismic selective screens can be tested using mice with a defined genetic back-ground, and with genes involved in DNA repair and cancer risk, inactivated or knocked out to produce "heterozygotes". By selecting cancers within or outside the selective screen of the immune system the effects of immunological surveillance on the risks associated with LOH of a cancer risk gene can be measured. By knocking out genes in the RAD 52 or RAD 6 pathways, the differential effects of low and high LET radiations, the effects of repair deficiencies on RBE, and dose rate effects can be measured in heterozygotes.

The interest in heterozygotes arises because they are expected to be present in the population at approximately four times the square root of the frequency of homozygotes, and because in terms of Knudson's multi-step theory of cancer risk, they have already inherited the first "hit" from their parents in the form of one abnormal cancer risk gene.

Clone 02 is abnormal, but in the same way that a heterozygous carrier of a Fanconi's anemia gene would be. As 1/77 of South African Afrikaans speakers are carriers of a Fanconi's anemia mutant gene, the Clone 02 results can be extrapolated to real human populations. It would, therefore, be expected that real humans would show linear non-threshold radiation-induced LOH and therefore potentially cancer risk.

RÉSUMÉ

Les données sur le Clone 02 de *Tradescantia* indiquent que les relations dose-effet sans seuil devraient être observées jusqu'aux doses et débits de dose les plus bas pour les rayonnements à faible transfert linéique d'énergie (TLE). Cette hypothèse devrait se vérifier également pour d'autres organismes vivants, même si le Clone 02 est radiosensible.

On arrive à la conclusion que le Clone 02 que la voie RAD 6 est partiellement déficiente pour la réparation des ponts entre les brins d'ADN et d'autres dommages liés à la perte d'information de codage, si l'on en juge par sa sensibilité à la fois au sulfonate d'éthylméthane et aux rayonnements ionisants. Les données sur le Clone 02 de *Tradescantia* qui montrent une relation linéaire sans seuil entre des événements génétiques somatiques reflètent en partie les carences des mécanismes de réparation de ce clone. On observe un taux plus élevé de réparation de l'ADN par des mécanismes de recombinaison chez le Clone 02 que chez la souche sauvage.

Deux catégories importantes de lésions de l'ADN sont induites par des rayonnements ionisants — les cassures de l'ADN double brin (DSB) qui sont réparées par des mécanismes de recombinaison, et les pertes d'information de codage (LCD) qui sont réparés par des mécanismes sujets à erreur, mais qui peuvent également être réparés par des mécanismes de recombinaison. À partir des données obtenues sur des levures, on sait qu'il existe deux voies différentes de réparation pour ces différents types de lésions, et que les conséquences génétiques somatiques sont différentes. Comme chez les levures, on peut obtenir des sections efficaces de rendement et les appliquer à l'endommagement et à la réparation de l'ADN chez *Tradescantia*.

On observe davantage d'événements génétiques visibles par lésion chez le Clone 02 que chez les souches sauvages. Chez un sous-clone obtenu par irradiation, le Clone 0106, lequel est plus variable que le Clone 02, on obtient encore plus d'événements par lésion. Ce clone dérivé et les expériences de croisement indiquent que le Clone 02 est hétérozygote, ou est « porteur » d'une version mutante d'un gène qui agit au niveau de la voie de réparation RAD 6 chez *Tradescantia*. Le Clone 02, par sa réponse à l'endommagement par les rayonnements ionisants, est donc semblable à un porteur de l'anémie de Fanconi dans une population humaine, alors que le Clone 0106 dérivé est semblable à un patient homozygote atteint de l'anémie de Fanconi.

On observe deux anomalies des courbes dose-réponse pour les événements liés à la perte d'hétérozygotie « rose », parce que le Clone 02 répare les deux types de lésions de l'ADN par recombinaison. Le Clone 02 présente une courbe dose-réponse linéaire pour les rayonnements à haut TLE. En partant de la même fréquence initiale de dommages, les types sauvages présentent une réponse sublinéaire. La réponse sublinéaire reflète une probabilité régulièrement décroissante que la variante « rose » soit générée en fonction de l'augmentation de la dose de rayonnement à fort TLE chez le type sauvage, mais non chez le Clone 02. Cette réponse régulièrement décroissante serait attendue pour la perte d'hétérozygocité chez des humains « sauvages ». Cela témoigne d'une proportion croissante de dommages de l'ADN réparés par des voies ne faisant pas appel à la recombinaison et/ou à une augmentation de la probabilité de la mort des cellules avec l'augmentation de la dose.

À de faibles doses et débits de dose de rayonnement à faible TLE, le Clone 02 présente une courbe dose-réponse linéaire, qui témoigne d'une probabilité de 1/16 qu'une lésion mène à une perte d'hétérozygocité, comparativement aux lésions causées par les rayonnements à haut TLE. Cette différence serait due : à des différences microdosimétriques dans le dépôt d'énergie et, par conséquent, dans l'endommagement de l'ADN par les rayonnements à faible en à fort TLE; au regroupement des lésions causées par les rayonnements à fort TLE sur la probabilité d'obtenir des effets génétiques; et à un aiquillage des lésions vers des systèmes de réparation qui ne produisent pas les effets étudiés chez les types sauvages. Même si l'on ne dispose pas d'observations à de très faibles doses et débits de dose chez les types sauvages, il n'y a pas de raison de supposer que la réponse linéaire sans seuil aux rayonnements à faible LET, chez le Clone 02 soit anormale. L'importance de l'événement génétique somatique qu'est la perte d'hétérozygocité est due au fait qu'elle reflète le risque de cancer chez les humains.

Les courbes de dose-réponse linéaire sans seuil à de faibles doses de TLE reflètent soit la probabilité que le clivage des structures de Holliday obtenues par recombinaison se fasse parfois selon une orientation très peu fréquante et produise une perte d'hétérozygocité, soit la probabilité que les lésions causées par rayonnements à faible TLE comprennent une petite proportion d'événements groupés comme ceux causés par les rayonnements à fort TLE ou les deux.

La probabilité de Poisson que deux lésions causées par des rayonnements à faible TLE soient provoquées au même endroit suggère que les effets du débit de dose dépendent de la coïncidence de lésions d'ADN au même endroit, et que la probabilité de perte d'hétérozygocité dépende de facteurs liés à la lésion et à sa réparation. Mais la pente de la relation dose-effet pour la perte de l'hétérozygocité chez le Clone 02 et toutes les autres souches ne se rapproche jamais de la valeur de +2 prédite dans le cas ou deux événements coïncident. Ces données indiquent que seule une combinaison particulière d'une perte de codage et d'une cassure double lorin coïncidentes mène, après réparation, à une perte d'hétérozygocité.

Tout indique que l'induction de dommages à l'ADN est linéaire aux doses les plus faibles. Les données indiquent que même aux doses et aux débits de dose les plus faibles de rayonnement à faible TLE, il y a toujours une probabilité de perte d'hétérozygocité causée par la réparation. Les effets sur le risque de cancer de cette perte d'hétérozygocité varieront en fonction du génotype du sujet et d'autres mécanismes sélectifs qui pourraient améliorer ou intensifier les effets de la perte d'hétérozygocité originale.

On peut vérifier les mécanismes de perte d'hétérozygocité causée par des rayonnements et les filtres sélectifs de l'organisme avec des souris possédant un profil génétique défini, et en inactivant ou inhibant des gènes intervenant dans la réparation de l'ADN et le risque de cancer de façon à produire des « hétérozygotes ». En sélectionnant des cancers à l'intérieur et à l'extérieur du filtre sélectif du système immunitaire, on peut mesurer les effets de la surveillance immunologique sur les risques associés à une perte d'hétérozygocité que présente un gène intervenant dans le risque de cancer. En inactivant des gènes dans les voies RAD 52 ou RAD 6, on peut mesurer la différence entre les effets causés par des rayonnements à faible ou fort TLE, les déficiences des méchanismes de réparation sur l'efficacité biologique relative, et les effets du débit de dose chez des hétérozygotes.

L'intérêt présenté par les hétérozygotes vient du fait que leur fréquence dans la population est environ quatre fois la racine carrée de celle des homozygotes, et parce que, selon la théorie multi-étape de Knudson pour le risque du cancer, les hétérozygotes ont hérité du premier « palier » de l'un de leurs parents sous la forme d'un gène anormal lié à un risque de cancer.

Le Clone 02 est anormal, mais de la même manière qu'un porteur hétérozygote du gène de l'anémie de Fanconi peut l'être. Un afrikaanophone sur 77 en Afrique du Sud est porteur d'un gène mutant de l'anémie de Fanconi; les résultats sur le Clone 02 peuvent donc être extrapolés à des populations humaines réelles. On devrait donc pouvoir s'attendre à ce que les humains présentent une perte d'hétérozygocité causée par des doses linéaires sans seuil de rayonnement et ainsi un risque de cancer potentiel.

Dans l'espèce humaine, on devrait donc pouvoir s'attendre à ce qu'il existe une relation linéaire sans seuil entre la pert d'hétérozygocité et les doses de rayonnement, et il en serait donc de même pour le risque potentiel de cancer.

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LINEAR DOSE RESPONSE CURVES IN FUNGI AND *TRADESCANTIA*

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SUMMARY

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All the evidence suggests that DNA damage induction is linear to the lowest doses. The evidence suggests that even at the lowest doses and dose rates of low LET radiation, there is a constant probability of LOH arising from repair. The effects on cancer risk of this LOH will depend upon the genotype of the individual, and further selective mechanisms which may ameliorate or intensify the effects of the original LOH event.

Radiation LOH mechanisms and organismic selective screens can be tested using mice with a defined genetic back-ground, and with genes involved in DNA repair and cancer risk, inactivated or knocked out to produce "heterozygotes". By selecting cancers within or outside the selective screen of the immune system the effects of immunological surveillance on the risks associated with LOH of a cancer risk gene can be measured. By knocking out genes in the RAD 52 or RAD 6 pathways, the differential effects of low and high LET radiations, the effects of repair deficiencies on RBE, and dose rate effects can be measured in heterozygotes.

The interest in heterozygotes arises because they are expected to be present in the population at approximately four times the square root of the frequency of homozygotes, and because in terms of Knudson's multi-step theory of cancer risk, they have already inherited the first "hit" from their parents in the form of one abnormal cancer risk gene.

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INTRODUCTION

The induction of genetic changes with low doses of ionizing radiation in the test organism *Tradescantia* Clone 02 [reviewed in Underbrink and Sparrow (1)] has been fundamental in establishing the linear non-threshold concept used as a basis for risk calculations in radiation protection (2, 3). However, Clone 02 has been described as being hypersensitive to ionizing radiation (1, 4).

The primary purpose of this review is to consider the possibility that the linear dose responses observed in Clone 02 is a reflection of abnormalities in its DNA damage repair. Is the linear dose response for somatic genetic changes observed in Clone 02 likely to be valid in other test organisms, and ultimately in humans, if it reflects an anomaly of DNA damage repair in Clone 02?

The broader purpose of this review is also to assemble and discuss the evidence from *Tradescantia* and other systems which bear on the question of the effects of DNA damage repair systems on the induction of somatic genetic "events", and their linearity of yield as a function of ionizing radiation dose. The specific context is to determine the extent to which variation in radiation-induced DNA lesions and in DNA repair parameters may lead to non-linearity in the generation of somatic genetic end-points.

Target theory (5) as it applies to specific lesions in DNA targets of defined sizes will be used as the underlying explanatory system. From effects in known targets of the size of a few base pairs to a few thousand base pairs in yeast (6, 7), the effects of repair systems on the generation of somatic genetic end-points will be extrapolated to the much larger targets in *Tradescantia* Clone 02 and related species (1, 4, 8).

The subject of this review is the effect of DNA repair systems on generating radiation-induced somatic genetic changes. Such somatic genetic changes are scored as surrogates for changes associated with cancer induction and progression.

VARIABLES IN THE RADIATION-INDUCED GENERATION OF SOMATIC GENETIC END-POINTS

Table1 lists variables affecting ionizing radiation-induced somatic genetic changes identified in this review. Some of the variables are discussed in more detail in the Appendices. If DNA damage is induced linearly with dose, and if damage repair systems generate end-points proportionately to the initial DNA damage repaired, then the prime expectation is to find linear, dose-related yields of somatic genetic end-points.

The variables which are important range from purely physical and microdosimetric to radiochemical, biochemical, and molecular biological. But beyond consideration at the DNA level, the consequences of biological organization and of the biological nature of living things must also be considered. Finally, it should be remembered that the numbers

of genotypes which have been studied is very small, and cannot represent the natural variability present in human and other populations.

Table 1: Variables affecting the generation of somatic genetic changes in irradiated living cells and organisms.

1. Types of spontaneous DNA lesions and their repair - Appendix 1.
2. DNA lesions and their radiation yields - DSB and LCD - Appendix 2 and Appendix 7.
3. Molecular models of repair of DNA lesions - Appendix 3.
4. How heterozygosity is lost, and the cancer implications of LOH - Appendix 4.
5. The linearity of LOH from gene conversion and other recombination systems in yeast and *Tradescantia* - Appendix 5.
6. Poisson lesion distributions in targets of various sizes and the probability of LOH - Appendix 6 and Appendix 7.
7. Effects of cell cycle and DNA damage on repair options.
8. Induction of repair systems by ionizing radiation.
9. Higher order selective systems as modifiers of the repair-based production of somatic genetic end-points - apoptosis and immune selection.
10. Human genetic variants which are expected to affect the yields of somatic genetic end-points and consequent radiogenic cancer risk. Human genetic "equivalents" of Clone 02.

An integrated understanding of these variables is needed to underpin radiation protection. The data from Clone 02 and from yeast are relevant to consideration of the first seven variables, which are largely concerned with DNA damage and repair, but as will be argued are not complete in defining all of the processes which determine radiation-induced cancer risks in mammals, and especially humans.

Variable 8 speaks to the induction of recombination repair. Because of the possibility of somatic genetic changes, the induction of recombination is not without potential biological cost to the organism. Variable 9 concerns the fact that terminally differentiated cells, such as those in *Tradescantia*, yeast, and even white blood cells, are not subject to the same levels of surveillance and selection as cells in tissues in mammals.

Finally, Variable 10 addresses the fact that humans are genetically unique, and that our experience with experimental genotypes is very limited, relative to the approximately five billion unique genotypes represented by human beings on earth.

CELLS, DNA AND REPAIR SYSTEMS

DNA is the hereditary molecule that encodes the information required for the development, growth, and control of the cells of the body, and for the sexual generation of offspring. The interest in effects of ionizing radiation on humans is largely because of the possibility that DNA damage will in some way lead to cancer. DNA repair is of interest because by-products of repair leads to loss of genetic information in somatic cells (loss of heterozygosity, or LOH). LOH is of interest because it is part of the cancer progression process, and if ionizing radiation induces LOH, radiation damage is mechanistically linked to cancer risk.

DNA is the same chemically and biochemically in bacteria, yeast, plants and animals. The systems which have evolved to monitor and improve DNA replication fidelity, and to repair DNA lesions will have direct analogues in all living systems. Biochemical identity means that what is learned in one system can be applied in another, at the same cellular and organismic organizational level.

Concepts developed by studying DNA damage and repair in lower organisms are valid across the whole spectrum of living organisms, as long as the organizational differences are identified and specifically taken into account. Yeast, because of its microbial unicellular growth, short cell cycle times, large populations and highly developed somatic genetic analytical systems, has marked advantages for the genetic, physiological and molecular analysis of DNA repair systems compared with any plant or animal system.

It is estimated (9) that about 6% of the 5884 genes of yeast have to do with DNA replication, recombination and repair (RRR). The common evolutionary relationships between DNA surveillance systems has led to progress in isolating the human, mouse, and plant DNA repair genes showing structural and functional homologies to the yeast DNA repair genes (e.g. 10, 11, 12). The recent complete sequencing of the *E. coli* genome is also relevant (13). In *E. coli*, about 5.5% of its 4,200 genes are thought to determine DNA replication, recombination and repair.

For yeast this means that about 350 genes specify all the enzymes and proteins which are required to replicate, repair, and recombine (RRR) the cellular DNA in both mitosis and meiosis. *E.coli* with its simpler life cycle needs only about 230 genes to carry out most of the same functions (meiosis is of course absent in this bacterium).

As DNA replication in man is biochemically the same as in lower eucaryotic organisms, one would expect about the same number of genes to be involved in specifying the biochemical systems as in lower organisms. That is, the biochemical details of DNA

replication, recombination and repair will remain the same, though higher order control systems may need to be genetically determined than, for example, in yeast.

Given a generous allowance for higher order control systems in mammals, it would still be expected that no more than about 600 to 1000 genes in the human genome will have the same replication, repair, and recombination functions as in yeast. This means that about 1% of the approximately 65,000 genes in the human genome are expected to have these functions.

But these functions are critical to the somatic maintenance and inheritance of genetic control mechanisms in the cells of a whole organism, where failure of cellular systems can lead to the death, and thus evolutionary failure of the whole organism. So the genes that control DNA RRR functions are critical in maintaining the functional state of the DNA over the life of the whole organism.

The full yeast genome DNA sequence (9) makes new levels of radiation biology possible. Analysing somatic genetic changes in terminally differentiated cells can therefore be based on an understanding of the functions of repair systems in yeast. Applying the concepts derived from yeast makes an explanation of the observations in Clone 02 and other *Tradescantia* strains possible.

DEFINITIONS

Somatic Genetic Changes

"Somatic genetic changes" need to be defined to avoid ambiguities of interpretation associated with many radiation biology studies. For example, the studies in *Tradescantia* commonly refer to "mutations" to describe the "somatic genetic changes" which are actually scored. But this is like referring to all radiation including UV, photons, neutrons, and other particles simply as radiation - the term is not explanatory.

"Somatic genetic changes" occur in cells within the body which do not get passed on to the next generation. The cells which make up the bodies of organisms are genetically determined and controlled, so genetic changes in cells which are passed on to daughter cells during cell division have undergone heritable, but not inheritable genetic changes.

"Inheritable genetic changes" occur in the sex cells in meiosis as a result of the normal processes of meiotic recombination, molecular accident, or exogenous DNA damage, and are inherited by the next generation. Thus, for example, new germ-line mutations, such as occur 70% of the time in Retinoblastoma, are inheritable, occur in the sperm or egg, and can be distinguished at the molecular level from "somatic genetic changes" which lead to LOH in the retinal tumors, and which reflect the sorts of mutational and LOH events we discuss below.

Meiosis is the process by which new combinations of the genes present in a genome are produced, to lead to unique new individuals after sexual reproduction. On average there are somewhat more than 30 recombination events per meiosis in humans, and, independently, all 23 pairs of chromosomes segregate at random, so on average each sperm or egg represents a combinatorial of one of about 2^{53} possible chromosomal DNA combinations. When two such gametes fuse, the resulting individual is genetically unique (14). One expects $(2^{53})^2$ potential combinations in the fusion of one sperm and one egg, or a total of a potential 8×10^{31} different combinations in all. This is an astronomical number; it says that each individual on earth is genetically unique.

But these changes are only of interest in permutating the genotype of a new individual; once the egg and sperm fuse, the only further genetic changes expected are the result of spontaneous rearrangements of DNA in cells leading to cellular, "somatic genetic changes". It is these "somatic genetic changes", some induced by ionizing radiation, which lead to cancer risk. The discussion of yeast, *Tradescantia*, and other systems in this review, therefore, centers exclusively on "somatic genetic changes".

Table 2: Classification of "Mutations" and "Genetic Events" in somatic cells at the molecular and chromosomal level. All these changes lead to loss of heterozygosity (LOH) of differing amounts of the cellular DNA coding machinery.

Class:	Name:	Definition:	Molecular Event:	Radiation Caused:
1	Mutation	Base pair change	Misrepair of LCD	Yes; RAD 6
2	Gene conv.	DNA transfer from homologue	Recomb. repair of DSB; LCD	Yes; RAD 52
3	Mit. Cross-over	Recomb. of homologues.	Holliday junction resolution	Yes; RAD 52
4	Interstitial del.	Recomb. within chromosome.	Non-homologous recomb.	Yes; V(D)J (SCID)
5	Translocation	Recomb. between non-homologues	Non-homologous recomb.	Yes; RAD 52 + V(D)J + End joining
6	Chromosome arm loss	Loss of material of one arm	Repair collision	Yes
7	Non-disjunc.	Loss of whole chrom.	Cell division before repair completion?	Yes; spindle poisons

Mutation

Table 2 above lists seven somatic genetic changes which come under the omnibus heading of "mutation" and which lead to loss of heterozygosity (LOH). A clear definition of the term "mutation" is required to avoid confusion. In this review the term "mutation"

means only changes in the base pair sequence in the coding portion of a gene. This includes transitions, transversions, deletions, insertions, and loss of RNA maturation functions if they occur at the base pair level.

Mutation at the DNA base pair level is a product of the yeast RAD 6 pathway, or its plant or mammalian equivalent, acting on spontaneous and induced DNA lesions (Appendices 1 and 2). The RAD 6 pathway is also called "error-prone repair" or "post-replication repair". Products of the RAD 6 pathway include induced base pair mutations as defined, and they arise in repair associated with DNA replication. The RAD 6 pathway is implicated in the misrepair of DNA interstrand cross-links and of bases damaged by alkylation (15, 16). The protein specified by the RAD 6 gene forms complexes including a polymerase which replicates past gaps in the DNA (17, 18). Because there is no coding information in a gap, insertion of a base will increase the odds of making an error, or a base pair mutation. The references listed refer to observations in both yeast and mammalian systems.

The target for mutation is individual base pairs in the DNA.

Mitotic Gene-conversion

"Mitotic gene-conversion" refers specifically to the physical transfer of genetic information from one chromosome to its homologue, within a gene. This leads to the elimination of one version of a gene and its replacement by another version. The physical transfer of coding DNA sequence from one chromosome to its homologue may be hundreds or thousands of base pairs long and is mediated by the mitotic recombination systems specified by the RAD 51 and RAD 52 system in yeast.

Thus, "mitotic gene-conversion" is not a result of the same repair system as base pair mutation, and should not be confused with it. In the case of "mitotic gene-conversion" the signal of recombination repair of DSB is the induction of new gene convertants (c.f. 6, 7). The radiation induction of gene convertants is held, in Appendix 2, to reflect the repair of actual DSB (or of DSB and LCD) induced in the base pairs of the DNA target.

The DNA target for gene conversion is of the order of thousands of base pairs (6, 7).

During recombinational repair physical DNA transfer occurs at points of contact of homologous (or sometimes non-homologous) chromosomes. This physical exchange needs to be resolved at the completion of the repair event.

A DSB is repaired recombinationally by turning it into two single strand breaks, which can easily be repaired. Using the homology of DNA sequence allows hydrogen bonding and repair DNA synthesis to occur on an unbroken DNA template. Holliday junctions are formed by physical exchange between the DNA templates involved in repair. These junctions need to be cleaved before mitosis to allow chromosome segregation. The

molecular cleavage of these junctions may lead to LOH by mitotic crossing-over, interstitial deletion, translocation, and other DSB repair-related events, as discussed in Appendices 3 and 4.

Mitotic Crossing-over

Christiansen (21) showed that in *T. hirsuticaulis* the "pink" end-point represents a mitotic cross-over. Assuming that this is true in Clone 02, the target can then be defined as those base pairs in the chromosome on which the "pink" and "blue" markers are located, between the "pink" gene and the center of the chromosome.

The "mitotic crossing-over" end-point is central in the *Tradescantia* Clone 02 data. Based on evidence presented by Christiansen (21) it is a reasonable conclusion that the bulk of "pink" events in Clone 02 actually reflect mitotic crossing-over between the "pink" gene copy and the center (centromere) of the chromosome it is located on. Mitotic crossing-over is a molecular result of the resolution of DNA repair intermediates established by the RAD 52 repair system.

The stamen hairs of *Tradescantia* flowers produce a file of "pink" cells which can be differentiated from the normal blue stamen hairs. As each stamen hair arises by the sequential division of one primordial cell, when the whole file has changed color this is because of a somatic genetic event induced in the initial primordial cell. In effect, there is a clone of cells which make up a stamen hair, and in which radiation-induced genetic changes can be scored easily, visually.

Evidence that the "pink" event actually reflects mitotic crossing-over event, and thus reflects the functioning of the RAD 52 process in *Tradescantia*, is discussed in References 20 and 21 and Appendices 1, 2 and 3.

The DNA target for mitotic crossing over is hundreds of thousands to millions of base pairs.

RADIATION SENSITIVE MUTATIONS, DNA DAMAGE AND REPAIR

The *Tradescantia* data is reduced from References 1 and 4 and is summarized in Table 3. The survival curves of Clone 02 after high or reduced dose rate low LET radiation show marked radiosensitivity relative to *T. blossfeldiana*, but do show a pronounced shoulder. At extremely low dose rates the shoulder might not be detectable, and cells might be killed linearly with dose.

The calculated numbers of DSB and other coincident lesions in Table 3 indicate that many lesions are required to kill a cell, and about three times as many, per base pair at risk, are required to kill *T. blossfeldiana* as are required to kill Clone 02. The contention

that Clone 02 is hypersensitive to ionizing radiation (1, 4) is true. But the comparison with the relative sensitivity of various yeast strains (below) indicates that there is still significant and substantial DNA damage repair capacity in Clone 02.

Table 3: Relative sensitivities of Clone 02 and *T. blossfeldiana* as a function of LET and dose rate [reduced from (1, 4)], lesions calculated under assumptions discussed in Appendices 1, 2 and 3. Irradiation at 20° C; X-rays at ~0.3 Gy/min high dose rate; 0.26-5.2 mGy/min intermediate dose rate; 0.43 MeV neutrons at ~5 mGy/min.

Tradescantia strains:	Repair defect:	LD_{37}:	DSB:	LCD:	Dose Rate
Clone 02	LCD	2.25 Gy	614	184	H.D.R.
T. blossfeldiana	None	11.4 Gy	2220	666	H.D.R.
Clone 02	LCD	0.25 Gy	90	87	0.43 MeV
Clone 02	LCD	4.50 Gy	1230	370	I.D.R.
T. blossfeldiana	None	26.0 Gy	5000	1,500	I.D.R.

Figure 1 (a) compares the survival after high dose rate, low LET radiation of *Tradescantia* Clone 02 with the related *T. blossfeldiana* species. Figure 1 (b) compares the relative survivals of the two strains after low dose rate, low LET radiation. Also on both Figures 1 (a) and 1 (b) is the dose response curve for Clone 02 irradiated with 0.43 MeV neutrons at approximately 5.0 mGy/min.

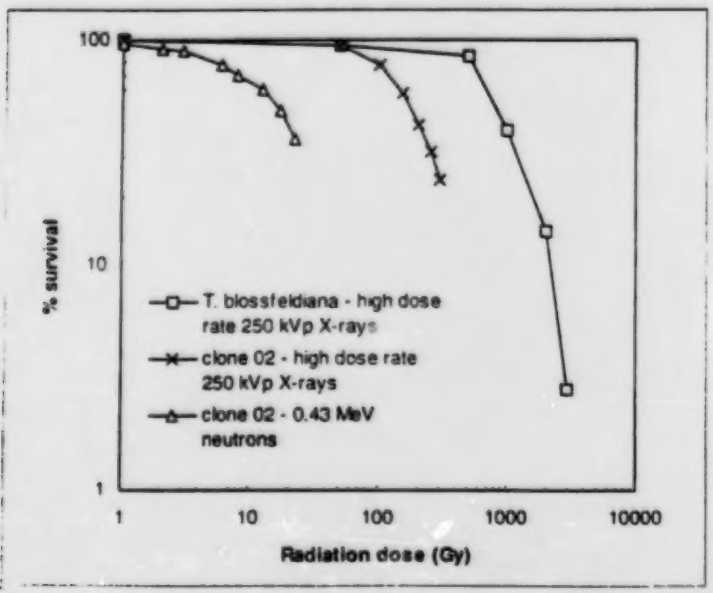

Figure 1 (a): High dose rate 250 kVp X-ray survival curves of Clone 02 (~0.3 Gy/min) compared with *T. blossfeldiana* (~2.63 Gy/min) and with 0.43 MeV neutrons (~5 mGy/min).

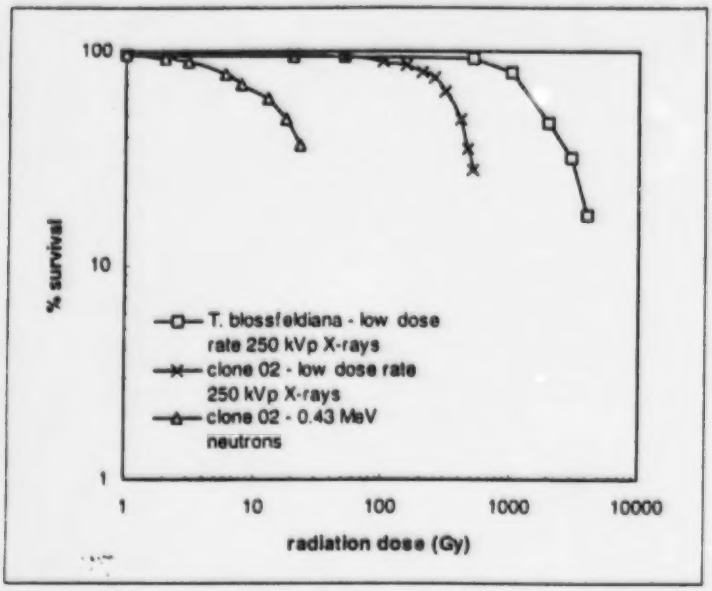

Figure 1 (b): Survival of Clone 02 after intermediate dose rate low LET irradiation compared with *T. blossfeldiana* and with 0.43 MeV neutrons on Clone 02.

The intermediate dose rates ranged between 0.26 mGy/min and 5.2 mGy/min for Clone 02, and 5.2 mGy/min to 41.7 mGy/min for *T. Blossfeldiana*.

The DDREF for Clone 02 in these experiments is about two-fold. The greatest DDREF observed in Clone 02 (38) is about six-fold comparing ^{137}Cs irradiation at approximately 0.3 Gy/min with the same radiation at approximately 0.04 mGy/min. High LET radiation is about 4.5 times as lethal as the high dose rate low LET radiation used above. The high dose rate low LET radiation is not as lethal per lesion as the high LET radiation.

References 2 and 3 discuss the possibility that for microdosimetric reasons the distribution of lesions differs between high and low LET radiation. The survival data suggests that high LET lesions are intrinsically more likely to be lethal, and that the lesions themselves cannot be repaired with the same efficiency as low LET lesions, so that fewer are required to induce an average of one lethal hit.

In Columns 4 and 5 of Table 3 it can be seen that the marked RBE for the induction of "pink" events reviewed below is also seen for hair cell killing by 0.43 MeV neutrons. While the RBE for killing is only about 10 comparing the intermediate dose rate killing with the 0.43 MeV neutrons, it is apparent below that the maximum RBE for "pink" induction is about 16 in the linear part of the dose response curve. Both these values, however, are dependent upon the low LET dose rate for their final RBE values.

IONIZING RADIATION SURVIVAL OF YEAST MUTANT DIPLOIDS

Figure 2 shows the survival data for yeast strains defective in one or other DNA repair system. In particular, the RAD 6 mutant is blocked in the repair of LCD, and the RAD 52 strain is abnormal in repair of DSB. Cells were irradiated at room temperature at 164 Gy/min in a ^{60}Co gammacell irradiator. There is a shoulder on the survival curve of wild-type; a shoulder of lesser magnitude on the survival curve of the RAD6 mutant, and linear killing of the RAD52 mutant with increasing dose. However, there seem to be two linear slopes on the RAD52 curve, suggesting that until the LD_{37} is attained, the RAD52 strain retains some residual repair capacity for low LET DNA lesions.

As is argued in Appendices 1 and 2, the major classes of DNA damage have dedicated repair systems which, in the case of the RAD 52 system, are cross active on LCD. This cross activity would reduce lethal lesion incidence by continued activity of the RAD 6 process, even while cells are accumulating lethal incidences of DSB.

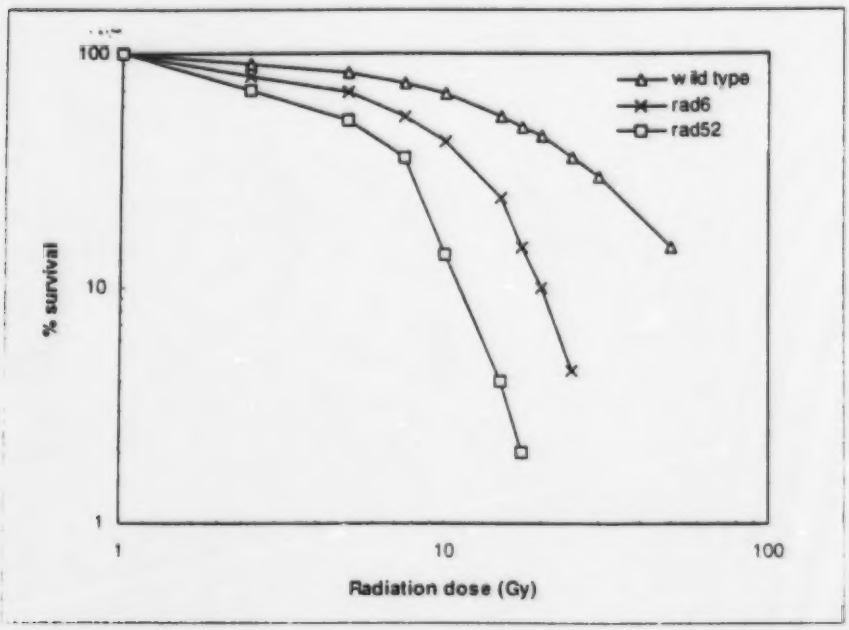

Figure 2: Radiation sensitivity of diploid yeast strains defective in none of the DNA repair systems, or in RAD 6 or RAD52. Data from unpublished work of P. Unrau and D. P. Morrison.

The yeast repair mutants inactivating the RAD 6, RAD 52 and RAD 51 systems, described in Table 4, vary in LD_{37} and are between 2 and 12 times as sensitive as the repair-normal wild-types.

Ionizing radiation sensitive mutants have been isolated from many organisms including bacteria (22), yeast and other fungi (23, 24, 25), plants (46), and animals (65). The sensitivity of such mutants means that DNA repair is a major factor in cell survival after ionizing radiation or other genotoxic DNA damage. Mutations abolishing the RAD 6 pathway also abolish radiation-induced mutation (26, 27).

The cross-sensitivity of RAD 6 mutants to ionizing radiation, UV, cross-linking and base damaging chemicals implies that these agents induce LCD. However, RAD 6 mutants do not abolish either mitotic gene-conversion or mitotic crossing-over. Recombination systems are expected to function independently if RAD 52 and RAD 6 deal with different DNA damage, or with the same damage by different repair processes. These concepts are expanded in Appendices 1,2 and 3.

Table 4: LD_{37} values for wild-type and yeast RAD mutant strains.

Strain:	Repair Defect:	LD_{37} :	DSB:	LCD:
Wild-type:	None	260 Gy	28	9
Rad 6	LCD	115 Gy	12.5	4
Rad 52	DSB	55 Gy	6	2
Rad 51	DSB	20 Gy	2	1

Mutations in the RAD 52 pathway are sensitive to agents which induce double strand breaks (DSB) (28). RAD 52 mutants do not generate radiation-induced mitotic gene-conversion, mitotic crossing-over, or meiotic gene-conversion (recombination within a gene in meiosis). Mutants in the RAD 51 and RAD 52 systems are mutators at the base pair level in mitotic cell growth [Morrison and Hastings (29); Morrison and Unrau, unpublished].

Because yeast cells lacking RAD 52 function are mutators, the implication is that the RAD 52 system in normal DNA replication acts to reduce spontaneous mutation rates (Unrau and Morrison, in preparation). The increased spontaneous mutation rate in RAD 51 and 52 mutants is because spontaneous lesions cannot be channeled through recombination, which is accurate at the base pair level, and thus exit via the RAD 6 error-prone repair system, with increased spontaneous mutation rates as a consequence.

If RAD 52 mutants cannot repair DSB, and cannot be induced to recombine to produce gene convertants, then a function of the RAD 52 repair process is the recombinational

repair of DSB. The production of gene-convertants is a signal of the activity within the converting gene of this repair process.

From sequencing the yeast genome it is known that about 60% of the genome is reduplicated (30). This facilitates two types of recombination repair - between strictly homologous DNA copies on homologous chromosomes, and by illegitimate recombination at DNA sequences which are very similar to each other. If RAD 51 is blocked in both systems, but RAD 52 is capable of illegitimate recombination repair, then the differences in radiation sensitivity between these key mutants in DSB repair could be explained.

References 31 to 33 give the various recombination-based models for the generation of gene convertants and the repair of DSB, discussed in more detail in Appendix 2.

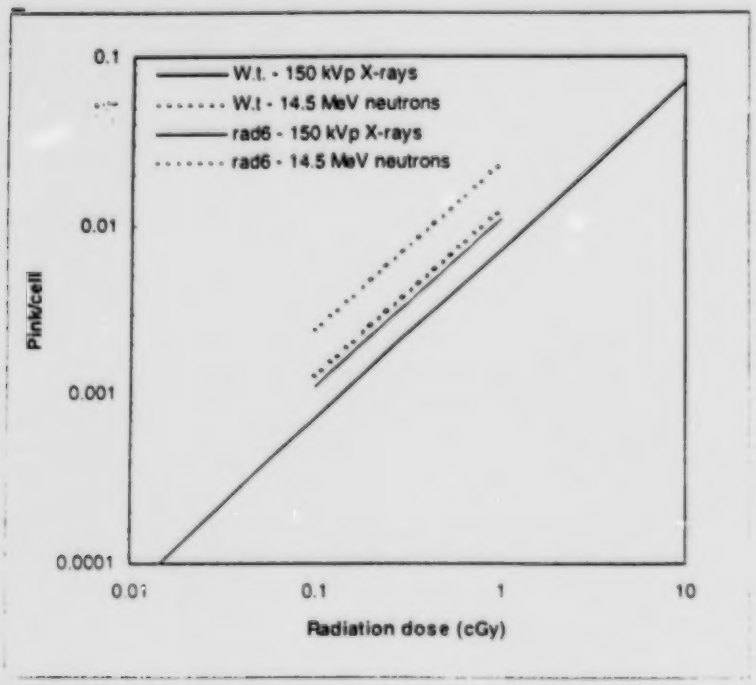

Figure 3: Gene conversion in oxically-irradiated wild-type and RAD 6 mutant yeast diploids using either 150 kVp X-rays, or 14.5 MeV neutrons; data from Refs. 7 and 27

Dose rates in the various experiments summarized above varied between 0.6 mGy/min and 164 Gy/min; no dose rate effects were established for mitotic gene conversion. Figure 3 gives the gene conversion dose response curves for wild-type and the RAD 6 mutant diploid yeast as a function of low and high LET irradiation. This data is clearly linear to the lowest doses, and at the lowest dose rates tested. Some of this data has been

reviewed in ACRP-11 and ACRP-18. The RAD 6 strain gene conversion data is reviewed here for the first time. The gene conversion target is estimated at 3600 base pairs [Unrau and Johnson (34)]. This physical target size is the same for both wild-type and the RAD 6 mutant diploid.

It is clear that the RBE of 14.5 MeV neutrons relative to low LET radiations, in a target of this size, is not more than 1.7 to 2.4. The size of the gene conversion target determines the range of RBE possible. A DSB or (in the RAD 6 mutant diploid) a DSB and/or LCD has to occur in the 3600 base pair gene conversion target to generate the end point.

If, as argued in Appendix 6, lesions need to coincide in time and space to produce a dose rate effect, it can be easily calculated that coincident lesions will vanishingly rarely be expected in such a small target.

It should be pointed out that there is no induction of ILV+ back mutations in the RAD 6 mutant strain; the data in Figure 4 is based on data from wild-type (in 6). The data in Figures 3 and 4 is the basis of the calculation of LCD yields given in Tables A2-1 and A2-2 in Appendix 2.

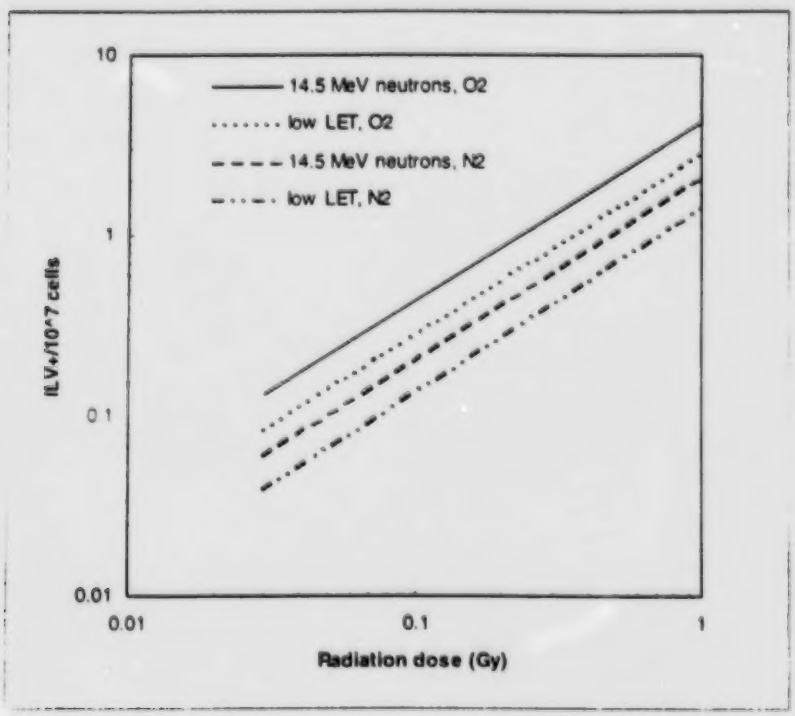

Figure 4: Ionizing-radiation induced back-mutation of the Ilv1-92 mutation in wild-type yeast as a function of radiation quality and irradiation conditions (0.03-1.14 Gy/min).

In Figure 4 back-mutation data from the wild-type yeast diploid is plotted for 150 kVp X-rays and 14.5 MeV neutrons under anoxic and oxic irradiation conditions, reduced from (7). The slopes of the dose response are linear with a slope of +1.0 on the log-log plot. The curve is linear with no threshold.

The curve reflects the probability that a DNA lesion will be induced in one of the bases which can be mutated or back-mutated to restore function to the Ilv1-92 mutant gene (the initial mutation in the strain). Based on DNA lesions, the data is taken to reflect the number of base pairs at risk, the number of LCD induced in those bases, and the probability of repair to lead to active metabolism of isoleucine and valine.

This implies that the RBE observed in a small target reflects the actual incidence of damage in the target. For base mutation, this reflects the probability of LCD in the bases which lead to a scored mutation, and the probability that the repair of the LCD leads to the mutational base pair change. Mutation induction in wild-type yeast is linear with dose (7 and Figure 4) and the observed data are consistent with lesions (LCD) in the 50-odd target base pairs leading ultimately to mutation.

These observations are extended at length in Appendices 1, 2, and 3.

The expectation that all primary lesions are induced linearly with dose is fundamental to an understanding of the effects of ionizing radiation (5). The data in (7) and (27) for the induction of mutation and gene conversion in yeast, at doses down to 1 cGy, are linear with no threshold. Clearly, the induction by ionizing radiation of lesions which either lead to gene conversion or mutation is linear with no hint of a threshold.

This means that, to the extent that the mutation and gene conversion end-points reflect the induction of DSB and LCD, induction is linear to the lowest doses of low and high LET radiation.

The fundamental linearity of damage induction means that if non-linearity is observed in data it must arise from processes active after the induction of the DNA damage.

So the question of the bulk of the *Tradescantia* data is, what are the shapes of the dose response curves for end-points that also reflect the action of DNA repair systems on radiation-induced DNA lesions?

TRADESCANTIA DOSE RESPONSE CURVES

The dose response relationships for somatic genetic end-points observed with *Tradescantia* have been discussed previously (2, 3). Figure 5 shows, on a log-log plot for ease of scale, dose response relationships for "pink" events in *Tradescantia* Clone 02 in

comparison with high and low LET irradiation-induced "pink" events in *T. subacaulis*, redrawn from Underbrink and Sparrow (1, 8).
The *T. subacaulis* data is used as a "wild-type" control, assuming that the "pink" target size is the same in the two clones so that differences in yields reflect differences in repair between the two clones.

On a log-log plot, a straight line with a slope of +1.0 reflects a linear process, which would be described by a straight line with a slope of +1.0 on a linear plot, like curve G1-2 [ARCP-18 (3)].

On a log-log plot, a straight line with a slope of less than +1.0 reflect a process in which the generation of an end-point decreases smoothly with dose. This would translate to a downward-inflected curve on a linear plot, somewhat like curve G-1-1, ACRP-18 (3), but not rising above curve G1-2 in that figure.

Finally, a log-log plot straight line with a positive slope, typical of low LET induction of somatic genetic changes for various end-points in *Tradescantia*, reflects a process in which the probability of generating an end-point increases smoothly with increasing dose. This would translate on a linear plot into a curve somewhat like either curve G-1-3 or G-1-4 in ACRP-18 (3). That is, it would be a "J" shaped dose response curve. A straight line with a slope of +2.0 on a log-log plot reflects a process in which two hits are required in a target to lead to an end-point.

In Figure 5, the linear dose response curves are back-extrapolated to see where the yield points intersect. The high and low LET curves for the two different clones coincide at doses well below the spontaneous yield of "pink" events, and thus below the "noise" level of the system. The implication is that at the coincidence point the absolute yields of "pink" clones is the same for the two strains, and at that dose the number of lesions required to induce the end-point is the same.

This is true if the "pink" target is the same size for both clones, and if lesions at the lowest doses have a high, constant probability of leading to the "pink" event. But observing the coincidence point is difficult because of the rate of spontaneous somatic genetic changes that occur in these strains.

In turn, spontaneous "pink" rates, vary between the strains and in many of the other clones tested, which suggests that the spontaneous somatic genetic events have a lot to do with the individual clone and its repair capabilities, and little or nothing to do with environmental factors such as ionizing radiation. This point is expanded in more detail for spontaneous somatic genetic events (mutation and gene conversion in yeast) in Appendix 1.

A more detailed comparison of the yields of "pink" clones relative to the expected yields of DSB and LCD in the targets by high and low LET radiation is given in Appendices 6

and 7. Figure 5 indicates for both Clone 02 and for *T. subacaulis* that the efficiency of "pink" induction is much lower for low than high LET radiation.

The different slopes for induction by high LET show that while the induction of the "pink" end-point is linear with a slope of +1.0 for high LET induction in Clone 02, it is sublinear in *T. subacaulis*. So for *T. subacaulis* Figure 5 shows that a decreasing proportion of high LET lesions in the "pink" target are being resolved to give rise to the "pink" end-point as dose increases.

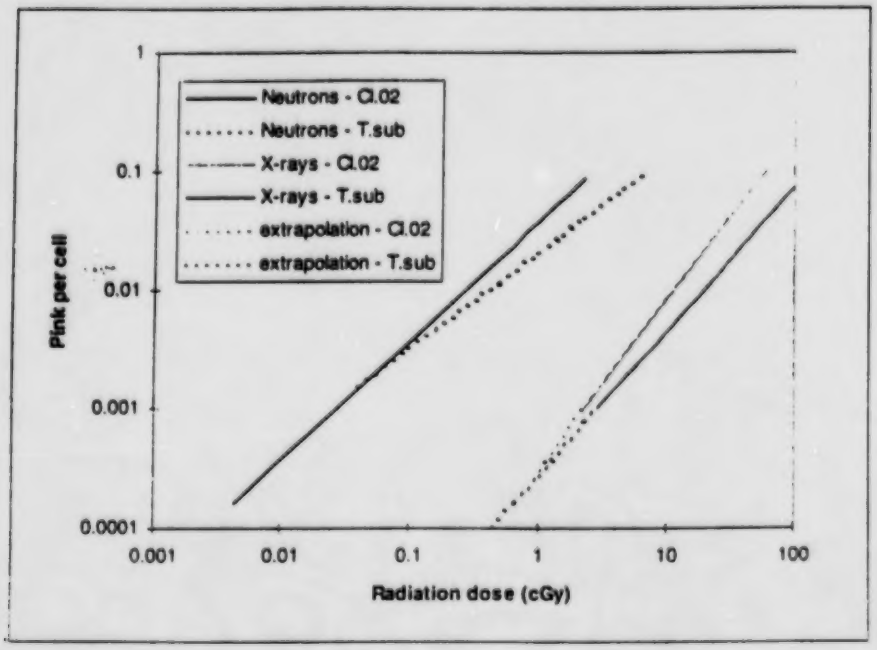

Figure 5: Induction of "pink" hairs in *Tradescantia* Clone 02 and *T. subacaulis* as a function of high (0.43 MeV neutron) and low (250 kVp X-ray) irradiation. Data reduced from Refs. 1 and 23: 20°C, 0.3 Gy/min X-rays and 5 mGy/min 0.43 MeV neutrons.

The bulk of induction studies on *Tradescantia* were carried out on Clone 02. Sparrow and colleagues scored other end-points both in stamen hairs and in other tissues of the plant. In such stamen hairs they were able to score changes in cell size and color as a function of radiation dose and quality. But they were unable to provide any somatic genetic analysis of the chromosomal and genetic changes they were observing. However, it can be assumed that color changes arise either from mitotic crossing-over (like their favored "pink" events) or from deletions and other gross chromosomal defects.

Figure 6 shows the induction of other genetic events Underbrink and Sparrow and their collaborators scored, after high and low LET radiation, in Clone 02, data reduced from References 1 and 8.

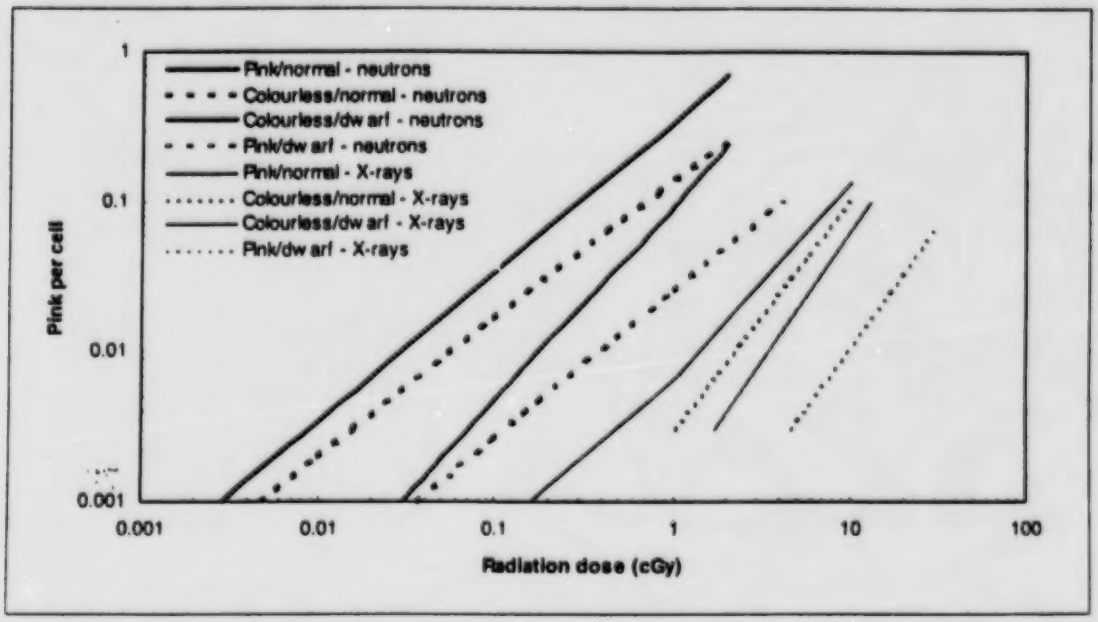

Figure 6. Induction in Clone 02 of somatic genetic changes with 0.43 MeV neutrons compared with 250 kVp X-rays. 20 ° C; 0.3 Gy/min X-rays; 5 mGy/min 0.43 MeV neutrons.

Table 5: Induction Slopes for Size and Color Variations in *Tradescantia subacaulis* Compared with Clone 02 (data reduced from Ref. 8).

Aberration:	X-rays:		0.43 MeV Neutrons:	
	T. subacaulis	Clone 02	*T. subacaulis*	Clone 02
Pink	+1.17**	+1.21**	+0.75**	+0.95
Colorless	+1.37**	+1.29*	+0.94	+0.91
Dwarf	+1.70*	+1.54**	+0.88**	+1.04
Giant	+1.24*	+1.21*	+0.69**	+0.84

* Significantly different from slope of +1.0 at 5% level.
** Significantly different from slope of +1.0 at 1% level.

From Table 5 it is clear that the expectation of generating a somatic genetic change in *Tradescantia* depends upon the strain, the radiation, and the target.

For Clone 02, high LET gives a slope of +1.0, while in *T. subacaulis* only "colorless" has a slope as high as +1.0. For *T. subacaulis* the expectation of a "pink" change never becomes either unity, or linear with dose. This translates into a yield curve which falls off smoothly and continuously with increasing dose.

For low LET radiations both Clone 02 and *T. subacaulis*, at doses above 6 cGy and dose rates of 0.29-0.31 Gy/min, have dose response curves with a slope significantly greater than +1.0, which translates to a "J" shaped curve from a log-log plot. But at all doses the absolute yields are less than for equivalent doses of 0.43 MeV neutrons.

To the extent that these "J" curves are offset in dose, because low LET radiation is less efficient at inducing genetic events than high LET, the curves could be mistaken for suggesting a threshold.

As expanded in Appendix 6, the suggestion is that for *T. subacaulis*, competition of LCD repair for high LET lesions removes potential somatic genetic events from the lesion pool by repairing the lesions in a way which does not yield the end-point. Clone 02, however, has linear dose responses with slopes not significantly different from +1.0 because competition for LCD does not occur, or only to a lesser extent. This suggests that because of the repair defect in Clone 02, more lesions are processed by recombination to give rise to somatic genetic end-points.

This would be expected if the repair of some classes of compound coincident lesion after high LET leads to the generation of the end-point, and other classes or groups of lesions do not. As discussed in Appendices 1 and 2, it is expected that high LET radiation induces both LCD and DSB in equal proportions in the majority of hits in a large target ($<10^5$ base pairs).

Because the yield of lesions per cell is expected to be linear with dose, and because the yield of "pink" events in Clone 02 is linear with dose, the absolute efficiencies of lesions from the various radiations can be compared. In Appendix 7, Figure A7-1 gives the expected yields of high LET lesions per cell, and the absolute efficiency of inducing "pink" events per lesion. Clone 02 is compared with *T. subacaulis*.

If the absolute efficiency of high LET hits in the "pink" target is assigned a value of 1.0, the size of the target, and the probability of a "pink" from a hit in that target can be calculated. The target is calculated to be about 2.6×10^8 base pairs in size.

In Appendix 7, Figure A7-2, the efficiency of low LET lesions at generating "pink" events is shown, and in Table A7-1 the relative efficiency of high and low LET radiations is compared. Data from Clone 0106 and *T. subacaulis* is included.

The conclusion is that, if the target is the same size, low LET lesions are and always remain absolutely less efficient at generating LOH events than high LET lesions. But the further conclusion is that the absolute efficiency of generating "pink" events depends crucially on the repair proficiency of the strain.

This fundamental principle would also apply in human and other mammalian LOH test systems.

In Figure 7, the number of single and double recombinagenic lesions (DSB and LCD) per standard "pink" target in Clone 02 are calculated for low LET and high LET, and then superimposed at equal numbers of lesions per target.

The dose response curves for the generation of "pink" hairs with either high or low LET radiation are then superimposed. The data show the absolutely higher efficiency of high LET lesions at the induction of "pink" events.

What is different about low and high LET DNA lesions that leads to such a marked difference in efficiency of generating a "pink" event? Based on considerations discussed in ACRP-18 (3), lesion density in the target as a function of LET, and differences in the molecular biology of repair of closely coincident lesions are expected to explain the observations. Recall that high LET lesions are significantly more lethal, as discussed above in Figures 1 (a) and 1 (b).

When the numbers of lesions in the "pink" target are normalized for low and high LET radiation, as is done in Figure 7, then the microdosimetric and molecular biological variables can start to be distinguished. Slopes of yields of the various end-points as a function of lesion density are discussed in more detail in Appendices 6 and 7.

Figure 7 is interesting because the inflection in slope of the low LET yields of "pink" intersects the line of yield of double lesions in the target. But the yield slope does not change significantly from +1.4, and certainly does not reach +2.0. This indicates that only some coincident lesions in the same target are repaired in such a way as to generate the "pink" end-points. A discussion of the combinations of lesion types (DSB + DSB; DSB + LCD, and LCD+LCD) is in Appendix 6.

Figure 7 indicates that at the lowest doses and dose rates, where (from Appendix 7) one high LET lesion in the target has a 77% chance of leading to a "pink" event, only about one low LET lesion in 20 in the target leads to a "pink" event. It is this dichotomy between the efficiency of the various sorts of lesions which needs to be explained to explain the concept of RBE. For this review it is sufficient to note only that Clone 02 yields "pink" events linearly with high LET dose, while the previous discussion of the *T. subacaulis* data indicates a sublinear yield.

Taken together, the data indicates that Clone 02 is more efficient at generating "pink" end-points per lesion than *T. subacaulis* and the argument is that this reflects the channeling of LCD lesions into recombinational, end-point producing repair pathways in Clone 02. In general, high LET lesions are both more efficient at generating LOH end points, and more lethal, than low LET lesions. Both microdosimetric and molecular biological considerations need to be understood to explain this aspect of radiation quality.

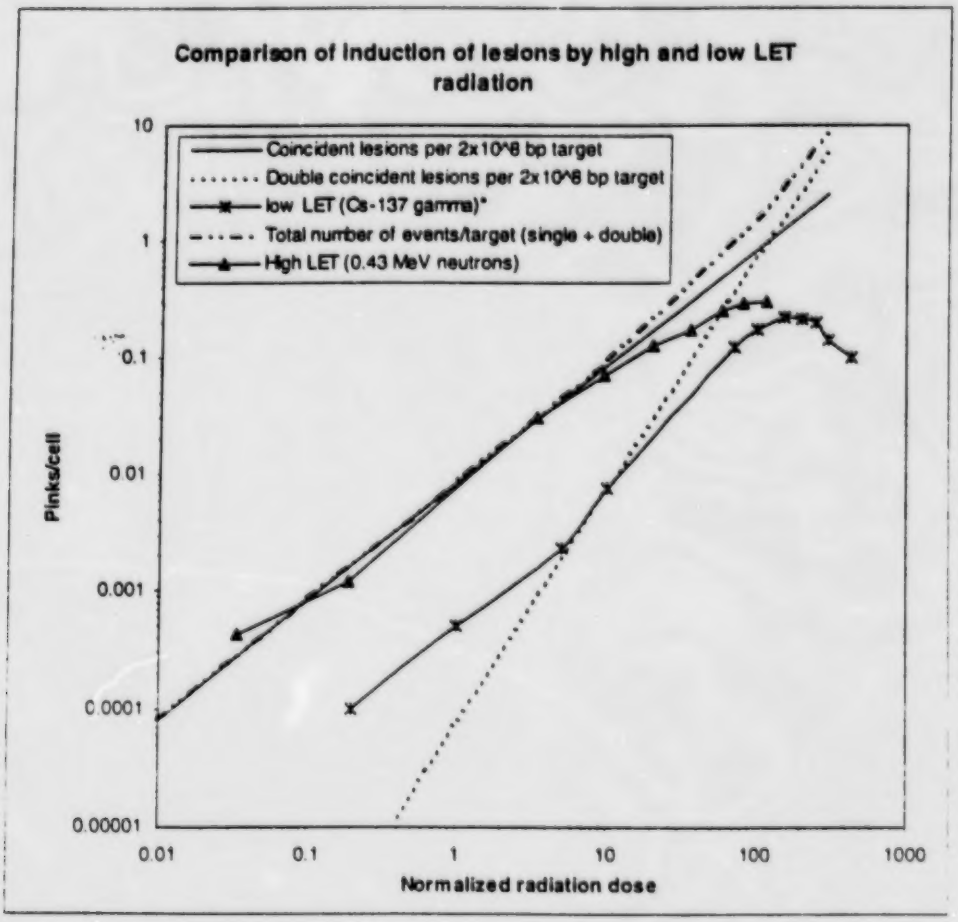

Figure 7: Yields of "pink" events at equal frequencies of lesions in the "pink" target with high and low LET radiation for Clone 02, data combined from Appendix 7, Figs. A7-3 and A7-4.

INTERPRETATION OF THE DATA FROM YEAST AND *TRADESCANTIA*

Yields of DSB and LCD lesions are linear with dose, with no threshold.

Coincident lesions lead, by the details of the molecular mechanisms of their repair, to somatic genetic LOH events in yeast and plants.

Wild-type organisms can deal with more such lesions than mutants, because:

1. There is cross-activity of the RAD 52 repair system on both DSB and LCD;

2. The RAD 6 system makes a major contribution to repair of LCD; and

3. There is the possibility that the two repair systems interact to render absolutely more lesions of both sorts repairable than by either system alone (49).

RAD 6 pathway mutants are radiation sensitive because radiation induces LCD and RAD 6 mutants cannot repair LCD.

Clone 02 shows many characteristics of the yeast RAD 6 mutation including: increased radiation sensitivity; sensitivity to other DNA damaging agents like EMS; and linear curves for the induction of most somatic genetic end-points with dose (34, 35).

Clone 02 also segregates a gene conferring even higher variability.

1. Mericle and Mericle (19) showed that the gene which leads to somatic genetic events was present in a single copy in Clone 02, and that they could cross Clone 02 offspring to get plants with either higher of lower rates of somatic genetic variability than Clone 02.

2. Studies by the Brookhaven group on Clone 0106 showed that after irradiation of Clone 02 they could obtain a somatic segregant (LOH for the variability gene?) after irradiation of the whole parental clone (34).

3. These observations suggest that Clone 02 is in fact a heterozygote or carrier for a partially inactive copy of a gene which in *Tradescantia* functions like RAD 6 in yeast.

4. Unfortunately, Clone 0106 is sterile, and no radiation studies were performed on the highly variable progeny the Mericles obtained.

I conclude that the linear dose response curves observed in Clone 02 with high LET radiation reflect the repair of both DSB and LCD via recombination.

At low doses of low LET radiation, the linear dose effect curves seen in Clone 02 may reflect absolutely higher yields of LOH events per initial DNA lesion, but there is no reason to suspect dose response curves in "wild-types" would be anything but linear.

In other *Tradescantia* species and clones, stamen hair color variants show that:

Mitotic crossing-over is the major underlying genetic process for generating these events (20, 21). The target for mitotic crossing-over is that DNA between the gene of interest and the centromere of the chromosome (21).

Lesions induced by high LET radiations are more efficient in generating LOH than lesions induced by low LET radiation in large targets.

Dose rate and dose effects in *Tradescantia* (36, 37, 38) with low LET radiation can be attributed to the occurrence within the same repair target or domain, at the same time, of more than one coincident lesion.

Low LET dose rate effects span about six-fold in the probability that the repair of DNA lesions will lead to the generation of somatic end-points (1, 35). At 0.04 mGy /min yields of events in Clone 02 are about 3 per 10^4 hairs at risk per cGy. At 3 Gy/min yields are about 16 per 10^4 per cGy. The DDREF is thus about 6 over a 10^5-fold range of variation in dose rate.

No similar data has been obtained for human radiation studies with low LET radiation. The dose rates used in studies on different *Tradescantia* species (usually about 0.3 Gy/ min) (1, 8, 38) lead to the expectation of compound coincident lesions (CCL)in the targets of interest, increasing the probability of generating an LOH event.

Such interactions are expected to lead to slopes of yields higher than +1 on a log-log plot. This is observed for doses above 6 cGy, and dose rates above 5 mGy/min, in Clone 02 (1); the low dose low LET observations on other *Tradescantia* strains typically show slopes of between +1.2 and +1.4 at doses well above 1 cGy (8, 35) at dose rates where the DDREF is expected to be least (~0.3 Gy/min).

Lea suggests (5) that a slope of +2 would be expected on a log-log plot for events which require two hits. None of the data from *Tradescantia* supports a slope greater than about +1.7 and the average for all dose response curves with positive slopes is about +1.4; this means that some kind of complex interaction is occurring which leads to a greater, but still not dose squared probability of end-point generation.

Because there are two types of recombinagenic lesions induced by ionizing radiation, and several different recombination repair systems, an alternative explanation is that when two lesions are induced in the same repair domain, only some combinations of repair events of some combination of such lesions lead to the end-points.

Calculations of the Poissonian probabilities of more than one lesion (Appendix 6, Figure 7), or combinations of the various lesion types in the same repair domain, may suggest a plausible mechanism for a higher probability of LOH when two lesions are being repaired. The induction and repair of two (possibly different) lesions in the same repair domain would be expected to show dose and dose rate dependence.

However, Lea (5) also suggests that no dose rate dependence is expected on microdosimetric grounds when the dose rates are low enough. This is supported by Underbrink and Sparrow's (1) analysis of very low dose rate low LET "pink" induction, where the slope falls to near +1.05 as the dose rate falls, even at doses which, at higher dose rates, show the interaction effect.

The implication for low dose linear non-threshold models is that at even the lowest doses and dose rates of low LET radiation there is still induction of LOH events. This may reflect either the underlying biochemical constraints on the resolution of repair events, or the Poissonian probability of two lesions being induced in the same repair domains irrespective of ultimate dose rate and possibly by the same ionization event. For low LET radiation the various end-points have yield slopes of +1.2 to +1.5 which is similar to curve 3, ACRP-18, Table G-1 (3).

For high LET radiation Clone 02 shows linear dose response curves down to 0.01 mGy, and is insensitive to dose rate over three orders of magnitude from 0.04 mGy/min to 50 mGy/min (1, 33). This is expected if high LET lesions occur in clusters such that interacting repair events are always expected (3). This would lead to much higher yields per Gy if interacting repair events themselves increase the probability of generating scoreable LOH events.

Other *Tradescantia* clones, and Clone 02 for "colorless" show a sublinear dose response relationship to low doses of high LET radiation, which is not precisely equal to curve 1, Table G-1, ACRP-18 (3). The curve is not the same as the ACRP example because the yield curves initiate at the same frequency and fall off smoothly from there. They never rise above a strictly linear dose response, so they are in fact sublinear.

Yield curves have slopes between +1.0 (for Clone 02), and +0.65 to +0.9 for "wild-type" *Tradescantia* irradiated with high LET radiation. This is explicable if the higher lesion density of LCD and DSB from high LET radiation lead to the channeling of some lesions (which induce "pink" events in Clone 02) into repair processes which do not yield "pink" events in the other Tradescantia strains.

The implication is that some lesion repair events lead with a low but constant probability to end-point generation in Clone 02 after low LET low dose irradiation. High LET repair events lead with much higher probability to the same recombinational outcome.

DATA FROM OTHER PLANTS

Mitotic recombination has been scored in other plant systems. Studies in Nicotiana (39, 40) in which color changes in cells on leaf surfaces are scored, also give generally linear dose responses. Figure 8 is a re-plot of data from the study of Fabries and Delpoux (41) in which this reviewer corrected the data for the contribution from spontaneous revertants. Corrected for spontaneous events, the curve is not significantly different from linear, and the slope not different from +1.0.

Other studies with Tobacco at higher dose rates of low LET irradiation (42) give dose response curves near the +1.4 observed in high dose rate studies of *Tradescantia*. These observations point to the generality of the low LET dose response curve being "J" shaped in a wide range of organisms and plants.

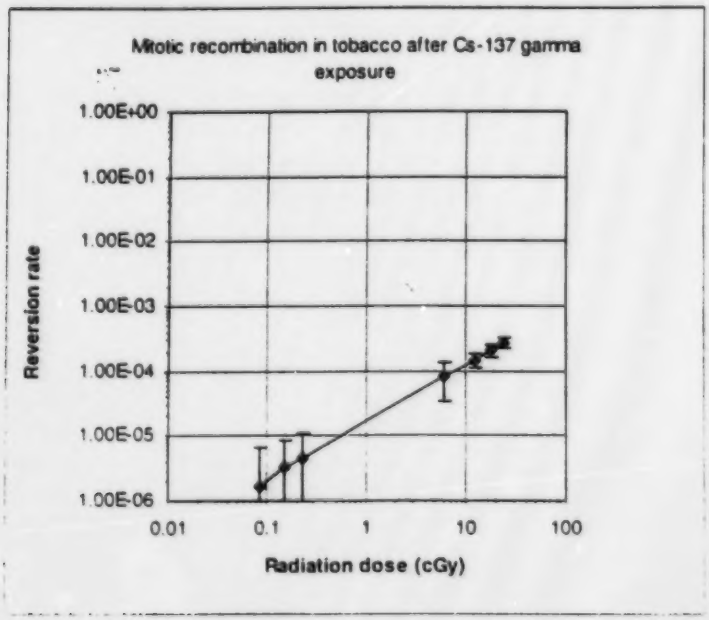

Figure 8: Dose response of induction of green cells from the greenish-yellow genetic heterozygotes by ionizing radiation, data reduced from (41) and corrected by subtraction of background recombination frequencies. Ambient temperature; dose rates 0.002 mGy/min to ~2 mGy/min.

Many dose response curves show an initial upwardly inflecting slope or "J" shape; this has nothing to do with radiation effects as it has been known since Muller's demonstration in 1930 (43, and see Appendix 1) that background radiation does not

explain spontaneous mutation rates, nor account for any significant fraction of spontaneous mutation in Drosophila.

The upwards inflection reflects the fact that at low doses spontaneous events are the majority, and only with increasing dose do induced events make up an increasing and ultimately the bulk of the scored events.

Other studies in tobacco have defined three LOH events induced by EMS treatment. This is done by recovering the phenotypically altered cells, inducing buds and thus whole plants in vitro, and analysing the resulting plants and their progeny. Dulieu (39) reports on gene mutations (base pair changes); true reversions, and deletions.
When twin-spots were analysed it was found that in tobacco the presence of closely similar chromosomes lead not only to "mitotic crossing-over", but to recombination between chromosome homeologues (related chromosomes from different species), with the loss of some genetic material. This is functionally a translocation system. However gamma-irradiation did yield reciprocal mitotic crossing-over products in *Arabidopsis* (44).

Plant genetic analysis has shifted to study of the small crucifer (cress family) *Arabidopsis thaliana*. The plant grows quickly, is amenable to genetic analysis, and has a genome ½ the size of Drosophila (1/50 the size of human and 1/500 the size of *Tradescantia*!). Mutants in the RAD 52 surveillance system have been obtained and described (45) and some which may be in the RAD 6 system have been isolated.

No data has been reported for the induction of genetic changes and mitotic crossing-over which may be relevant to this review, using the somatic genetic systems which are available in plants together with low dose ionizing radiation studies. However, it is clear that studies of the linearity of LOH events in various repair defective mutant lines of Arabidopsis could be commissioned.

RELEVANCE OF THE YEAST AND *TRADESCANTIA* DATA TO HUMAN CANCER RISK

Yeast studies on irradiation of synchronized cultures of haploid and diploid cells have been performed (46). The cell cycle is broken up into G_1 phase before the DNA replicates, and the G_2 phase after DNA replication. In a haploid in G_1, there is thus only one copy of the DNA, but in a diploid (like humans) each parent will have donated one copy, so the DNA in G_1 is redundant.

The G_2 phase is after DNA replication, so for a haploid there are two copies of DNA, and for a diploid there are four. Thus, recombination can occur in both G_1 and G_2 in diploids, because there are redundant DNA copies, but only in G_2 in haploids. The preferred substrate for DSB repair in G_2 seems to be to use sister-chromatids as the source of

genetic code for the repair intermediate. Sister chromatids are genetically identical so no LOH can be detected.

The initial studies on the induction of recombination repair systems was carried out in smut (48, 49) and in yeast (50). These studies indicated that cells could be induced to recombine, and that their RAD 52 systems could be enhanced by prior radiation doses. A requirement for protein synthesis was established (51) for induction.

The brilliant yeast recombination induction studies of Roman and Fabre (52) indicate that the induction of recombination repair does not require DNA damage as such. Evidence has been obtained that a block in DNA synthesis (53) is sufficient to induce recombination.

These studies showed that there are other cellular targets, or signals from damaged DNA, that act as recombination inducers. No such evidence has been obtained in *Tradescantia*, but evidence is accumulating that similar processes occur in some mammalian cell systems (54).

However, because the plant and yeast systems do not involve tissues and organs in which cells with LOH are subject to further levels of surveillance (55) including by the mammalian immune system, it is not likely that further compilations of LOH data in these terminally differentiated systems will be very informative to the linear non-threshold debate.

Because all the data on LOH induction and the repair of DNA lesions has been derived from a very small number of tested genomes, a much greater effort will be needed to define the limits of variability of humans in their LOH responses to ionizing radiation. Aside from the coincidence that Clone 02 seems to be a "carrier" of a mutant DNA repair gene, the evidence does not speak to the distribution of defective DNA repair genes in the individuals who make up human populations.

Preliminary data exists (56, 57, 58) which indicates that the range of variation in radiation sensitivity of "normal" humans (or at least, cell lines derived from them) may be as great as seven-fold. This amount of variation is much greater than any which could be attributed to uncertainties in, for example, dosimetry. So two major areas of uncertainty remain to be addressed before the generality of the linear non-threshold concepts are fully validated; the effects of organismic surveillance systems, and the range of genetic heterozygosity and hence risk of LOH in human individuals.

Table 6: Effects of radiation and repair variables on dose response curves in yeast and *Tradescantia* on the linear non-threshold generation of somatic genetic end-points.

1. Lesion types: DSB and LCD
2. Lesion yields: linear with dose
3. DNA repair: DSB and LCD by two systems; RAD 6 LCD specific; RAD 52 cross-active on LCD and DSB; saturation-> death
4. End-point generation: Probability approximately unity high LET; dependent on Poissonian + repair saturation + target size for low LET
5. Target sizes: Small - unit DSB or LCD in target base pairs; Large - unit end-point yields with high LET; low end-point yields low LET
6. Lesion-repair interactions: complex; not two hit kinetics
7. Cell cycle effects: G_1/S recombination; G_2 sister chromatids
8. Radiation blocks DNA synthesis; blocked DNA synthesis leads to recombination induction; more recombination/lesion
9. Yeast and *Tradescantia*: no cellular selection mechanisms
10. Limited range of yeast and *Tradescantia* genomes tested; linear, but yields/lesion vary by 2-10-fold and spontaneous rates by 2-20-fold. Human ranges as large.

TESTING THE IMPLICATIONS OF BIOLOGICAL VARIABLES ON DOSE RESPONSES IN HUMANS AND OTHER MAMMALS

Selective Screens in Mammals

The influence of organismic selective screens in reducing the cancer risk of LOH cells produced by ionizing radiation can be tested directly in "gene knock-out" mice. In such mice, genes implicated in cancer and radiogenic cancer risk can be knocked out so that the test animals are heterozygotes. Ionizing radiation or other generators of LOH can then be used to induce tumors.

To test the effects of immune system surveillance, one could compare the dose effectiveness of ionizing radiation at inducing gliomas (brain cancers) (59) with, for example, the efficiency of induction of cancers in p53 knock-out mice (p53-/p53-) (60). The brain tumors are inside the "brain-blood" barrier, which excludes molecules the size of antibodies. Thus, little or no immune surveillance would be expected. The testicular tumors and lymphomas characteristic of the p53+/p53- knock-out heterozygotes are, however, fully subject to immune surveillance and thus, would be expected to be selected against by the immune system. Suitable mouse model systems can be found to test these factors.

Mutations (61) in the p53 gene and the severe combined immune deficiency (SCID) gene interact at the genetic cancer risk level so that p53-/- SCID/SCID mice develop tumors earlier than their p53-/- relatives. This suggests that the p53 gene product interacts with

components of the immune system, and that the absence of this interaction leads to loss of some degree of normal cellular surveillance.

Similarly, mice in which components of the apoptotic surveillance systems have been mutated would provide insights into the importance of this system in reducing the effects of DNA lesions which may lead to LOH. The key is that mammalian genetics has progressed to the point that such experiments are no longer inconceivable, and the strains have the advantages that, except for the gene of interest, the remainder of the genotype is controlled and constant within an experimental test-cross. This identity goes so far as litters sharing the same uterine environment, and with suitable crosses, sharing more than 95% of their genes in common.

LINEARITY OF DOSE RESPONSE IN WHOLE MICE AND IN WHITE BLOOD CELLS

A specific test of both linearity of dose response and of cancer induction in mice has been proposed. The locus involved, p53, is a gene situated about half-way along chromosome 11 of the mouse. Thus, the chromosome presents a LOH target of about 3×10^8 bp. The calculations about mice at risk are based on the assumed effectiveness observed in *Tradescantia*, Appendix 2. There are expected to be about 1.8 lesions per cell per in this chromosome arm per Gy. But for gamma, only about 1 lesion in 150 in wild-type *Tradescantia* leads to MCO, so the expectation is for a 1% MCO rate per chromosome 11 per Gy, of which 50% may lose heterozygosity for a knock-out mutation of the p53 gene to go from p53+/p53- to p53-/p53-.

One cell in 200 after 1 Gy of whole body gamma irradiation is expected to show LOH for p53, but if this dose leads to two primary tumors per mouse of 10^{11} cells, then there are between three and four steps of this size required for full cancer induction and progression. This is well within the expectations of the Knudson multi-step cancer progression hypothesis. Methods of detecting the molecular events which give rise to Knudson's second hit (LOH) in heterozygotes are being continuously refined (62).

However, there are direct tests of the hypothesis of the induction of 1 LOH event per 200 chromosome 11 targets at risk per Gy in the mouse p53+/p53- system. This is because the system also includes specific assays for LOH of the wild-type chromosome arm, which depend upon PCR assays to associate LOH events with molecular changes in chromosome 11 and at the p53 locus in p53+/- heterozygous mice. Briefly the assay is to look at the molecular events associated with radiation-induced tumors in tumor cells, and at chromosomal LOH events which produce the p53-/p53- cell phenotype in tumors. A pattern of specific types of chromosomal LOH associated with ionizing radiation would then serve to classify the molecular nature of ionizing radiation-induced cancer risk.

There are about 10^7 terminally differentiated white blood cells in a mouse. If 1/200 have MCO leading to LOH, there should be 1000 events in 10^5 WBC which can be obtained

from 0.1 ml of mouse blood. Dose response curves down to very low doses can be obtained if two conditions are met: flow cytometry to separate p53-/p53- LOH cells from the bulk p53+/p53- heterozygous cells; and analysis of the molecular events leading to LOH of the p53+ gene in the p53-/p53- cells.

If the LOH events are sorted into the seven possible classes, 0.1 mL of mouse blood might be enough to generate the data to classify radiogenic LOH events to a great degree of certainty. The only assumption is that each LOH event which is scored is independent of any other; this is not quite true if early LOH stem cells have a selective value large enough to make them produce a large part of the population at the time of scoring.

The further extension is to validate scoring LOH in WBC as a surrogate for radiation-induced tumors in these mice. This would make possible very low dose studies of linearity of cancer induction in mice.

RBE OF CANCER INDUCTION IN MICE

The other test which springs naturally from the mouse LOH systems is to test the actual RBE of 14.5 MeV neutrons for cancer induction in normal and p53+/p53- mice. If there is in fact a 10-fold higher yield of mitotic cross-overs in a target the size of mouse chromosome 11, this should be obvious on examining a small number of white-blood cells. The hypothesis that high LET radiations have a higher efficiency of induction of LOH end-points can be tested directly in a mouse system. Testing the actual RBE for cancer induction could also be performed directly in p53+/p53- heterozygous mice in comparison with high and low dose rate, low LET gamma rays.

GENETIC VARIATION IN HUMANS AND CANCER RISK

No test can yet take account of the extremes of genetic variation of human individuals. For mice, yeast, *Tradescantia* and humans the range of genetic variation tested is minute. Each human (14) has a unique genotype (barring identical twins). The proposed experiments on mice can only take account of a minute part of the genetic range of variation found in living, functioning humans (or mice).

Table 7 lists some of the genes associated with specific cancers, and with LOH, which are known to segregate in human populations. Each or any of these may be cancer risk factors on irradiation.

Table 7: Specific Cancers Associated with Loss of Heterozygosity (LOH) of Cancer Risk Genes.

Cancer:	OMIM#	Chromosome Location:	
Familial Breast Cancer	114480	17p33.3[a]	
Breast Cancer Type 1	113705	17q21	
Adenomatous Polyposis Colon	175100	5q21	
Breast Cancer p53 Regulator	113721	17p13.3	
Bladder Cancer	109800	13q14.1	
Ovarian Tumor	167000	6q26	
Cancer of Colon	114500	17p13.1	
Breast Cancer 2, Early Onset	600185	13q12	
Colon Cancer, Non-polyposis	120436	3p21.3	E.coli repair
Multiple Endocrine Neoplasia	171400	10q11.2	
Papillary Thyroid Carcinoma	188550	10q11.2	
Testicular Tumors	273300	12q22	
Retinoblastoma	180200	13q14	
Neuroblastoma	256700	1p36.3	
Tumor Susceptibility Gene	601387	11p15.2	
Basal Cell Nevus Syndrome	109400[b]	9q22.3	

[a] Chromosome locations identify the chromosomal fragment lost or altered in a cancer, and helps define the gene location. p = the short arm; q = the long arm of the homologue numbered.

[b] We have identified more than 800 genetic syndromes in Online Mendelian Inheritance in Man (OMIM) which define genetic elements of cancer risk, progression, or cell surface change [Doerffer and Unrau, Health Physics (67)]. More than 500 of these syndromes are already associated with DNA sequences so that molecular analysis can be performed.

A brief survey of Med. Line, an on-line database cross referencing some seven million biomedical publications, turned up 726 references to loss of heterozygosity (LOH) in specific tumor and cancer cells. Table 7 lists some of the genetic syndromes associated

with cancer risk, and characterized by LOH for genes which have been located in the human genome. LOH itself is thus a hallmark of the cancer process.

Molecular screening tools for finding heterozygotes in the most common such genes are being developed (63). Isolation of gene products and the selective analysis of those which have base sequence variations will be commonplace within the next five years, and it is these assays which will provide the information about population and individual variation upon which an individual-based radiation protection system may be built.

This review makes the argument that Clone 02 is heterozygous for a RAD 6 repair gene, which affect the yields of somatic genetic end-points, and their dose response curves. But that fact does not invalidate the insights into dose effects based on the Clone 02 data.

There are at least 500 genes in humans which affect DNA replication, recombination and repair, and we do not know how many heterozygous carriers exist in the population, having inherited the first "hit" in Knudson's multi-hit cancer risk progression.

Indeed, we have argued [Doerffer and Unrau (67)] that it is largely amongst those humans who already carry a variant of a cancer risk gene that cancer risk is distributed. This argument is supported by Darlington's analysis (14), that only about 20% of two pack a day smokers die of lung cancer. One implication is that the lung cancer sub-population is already "primed", by carrying a cancer risk gene, to have a higher lung cancer risk than their smoking colleagues who are not heterozygous for a cancer risk gene.

POPULATION GENETICS

The argument has been made that Clone 02 of *Tradescantia* is abnormal in a repair process which acts on DNA damage induced by ionizing radiation and other mutagens. This spectrum of sensitivity is similar to that observed in yeast RAD 6 mutants, and in human Fanconi's anemia patient cell lines. The role of the RAD 6 processes in repair of DNA damage, from base alkylations to interstrand cross-links has been elucidated in yeast (see above; Appendices 2 and 3).

The suggestion that Clone 02 is a heterozygote for a RAD 6 pathway gene of *Tradescantia*, based on the genetic analysis of the Mericles (19), and on the derivation of Clone 0106 by ionizing radiation from Clone 02 (34) leads to the hypothesis that its behavior may closely mimic that of human Fanconi's anemia heterozygotes.

Owing to founder effect, the South African Afrikaans speaking population has a rate of heterozygosity for the Fanconi's anemia complementation group A mutation of about 1.7% (64). The radiation responses of this significant population group have not been tested, but in principle they could be.

An FA knock-out mouse model is available (65) and could be used in direct tests to measure the effects of ionizing radiation on inducing LOH in heterozygotes which should be functionally similar to Clone 02. It should be pointed out that FA fibroblasts have been shown to be hyperrecombinational, which would be expected by analogy with RAD 6 mutants of yeast (66).

The model envisaged would use FA heterozygous mice as surrogates for human FA gene carriers to see if low dose cancer risk is detectably altered in carriers. Because many LOH events can be scored per mouse, and because cancer risk should be related in some way to LOH, such experiments would serve to better define the importance of genetic risk factors in radiogenic cancer risk. This type of experiment needs to be combined with epidemiological studies of defined human populations to determine which cancer risk genes are segregating in which populations.

Taken together, laboratory studies of a selection of cancer risk genes in mice, and population studies of the actual distribution of cancer risk genes in populations may reduce uncertainties of the degree to which cancer in populations may actually be attributed to ionizing radiation.

ACKNOWLEDGMENTS

The helpful readings of the manuscript by Dave Myers has been appreciated. Discussions with Tony Waker about the application of data from yeast to *Tradescantia* has helped clarify some of the relationships between dose, dose rate, LET microdosimetry, and repair. Sara Carlisle reduced the diagrams to electronic form. The support of Bob McElroy during the preparation of the manuscript is also acknowledged.

The opinions expressed in this document are entirely my own, and do not represent the views of AECL in any official capacity.

A NOTE ON THE REFERENCES

The papers on *Tradescantia* were a kind gift of the Brookhaven Labs where the work was done. The modern (post 1975) references were retrieved from the National Center for Biotechnology Information from their web site on: http://www.ncbi.nlm.nih.gov/Entrez/ or from hard copies. On-line Mendelian Inheritance in Man (OMIM) references were retrieved from OMIM at: http://www.ncbi.nlm.nih.gov/OMIM/.

In such a review it is impossible to do justice to the many millions of person-years of research which underpin our understanding of the processes of DNA damage and repair. It is hoped that the references cited will serve as an entry to the relevant literature.

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APPENDIX 1

MECHANISMS OF THE REPAIR OF SPONTANEOUS AND IONIZING-RADIATION INDUCED DNA DAMAGE

Molecular mechanisms of DNA damage and repair have been proposed. These are based on ionizing radiation sensitivity of yeast strains mutant in various DNA surveillance systems. As outlined in the main text, mutations in three major DNA surveillance systems are considered. Figure 1 shows the various types of DNA damage which are envisaged, in this case induced by ionizing radiation or some other genotoxin. This appendix will argue that spontaneous lesions functionally equivalent to those induced by ionizing radiation occur all the time, and provide the major substrate for the various cellular repair mechanisms. The action of these repair systems leads, as a by-product of DNA damage and repair, to the end points used as an indicator of the initial DNA lesions.

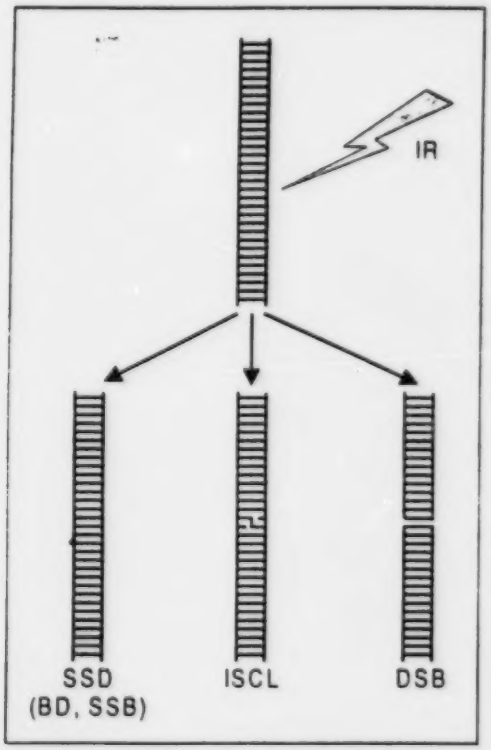


Figure A1-1 is a cartoon of the three major classes of DNA damage which occur to DNA either spontaneously, or after genotoxic damage. Crudely, the damage can be confined to one DNA strand and repaired by reference to the alternative strand as a template. Alternatively, DNA damage can lead to the loss of coding information on one or both strands as damaged bases are removed (loss of coding damage - LCD), leaving a "hole"

in the template. And finally, coincident scission of the DNA phosphodiester backbone leads to the loss of structural integrity of the DNA template by causing a double strand break (DSB).

Because the three major classes of DNA damage have different implications for repair, and for the generation of genetic end-points as a by-product of repair, the damage control systems will be described in more detail. In essence, damage in one DNA strand can be repaired by reference to the other, reflecting the fundamental template nature of the DNA molecule. Such damage includes UV-induced pyrimidine dimers, and some of the various classes of base damage and single strand breaks associated with ionizing radiation damage. Repair of this class of damage is largely the result of the action of the RAD 3 system.

Two sorts of coincident lesions can be induced in DNA - DNA damage which results in loss of coding information ((LCD or Loss of Coding Damage), e.g. inter-strand DNA cross-links (ISCL)), and double strand breaks (DSB). The evidence for the induction of LCD by ionizing radiation is largely circumstantial, and consists of the observation that yeast mutants abnormal in LCD repair cannot be induced to mutate with radiation or most chemicals (A1-1, A1-2), are sensitive to ISCL-inducing chemicals such as psoralens and mitomycin C(A1-3), are sensitive to UV and ionizing radiation (implicating these agents in LCD induction)(A1-4) and are hyper-recombinationally proficient (A1-5,A1-6). This would be expected if the LCD and DSB repair systems were genetically independent.

This would also be a propitious time to point out that Fanconi's anemia patients have cells which are sensitive to MMC, UV and IR and other chemicals (A1-7, A1-8, A1-9) and which show chromosome instability because they are hyperrecombinational (A1-10). It should further be pointed out that FA carriers comprise up to 1.7% of the South Afrikaans speaking South African population owing to founder effect (A1-11).

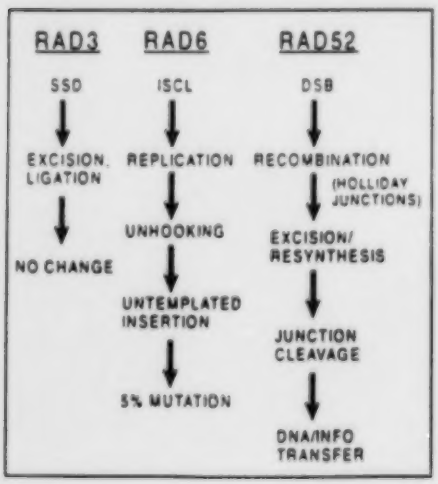


Figure A1-2 above introduces the three major DNA damage surveillance systems and summarizes the types of damage they deal with, and the genetic consequences.

Repair of DSB occurs by recombinational means, by using another DNA molecule as a structural framework for turning two coincident strand breaks into two single strand breaks(A1-12). Alternative DSB repair mechanisms include non-homologous repair (A1-13) and end-rejoining (A1-14). Homologous recombination is discussed in more detail below.

TableA1-1 shows the effects on spontaneous gene conversion and back mutation of mutations inactivating three different RAD systems. These end-points are induced linearly with dose in yeast (Tables 3; 4; Appendix 2); the data is of the effects of the RAD mutants on spontaneous generation during normal cell growth. The data reflects the means and standard errors from 20 fluctuations tests for each yeast strain.

The data shows that mutations in any of the three DNA surveillance systems lead to significant increases in mutation, gene conversion, or both in the absence of exogenous radiation. This implies that the normal function of these systems is to improve the fidelity of DNA replication.

TableA1-1: Spontaneous gene conversion and mutation rates in yeast strains normal or defective for various DNA repair systems: incubation at 30°C. TRP+ = mitotic gene conversion; ILV+ = spontaneous mutation.

Strain:	Defect:	Radn. Sens:	TRP+/10^6 Cells	ILV+/10^7 Cells
Wild-type	None	Not	5.4 +/- 0.5	2.6 +/- 0.4
rad 3	UV-dimers	UV; not I.R.	14.6 +/- 1.1	3.5 +/- 0.4
rad 6	LCD	UV + I.R.	39.8 +/- 3.5	1.5 +/- 0.4
rad 52	DSB	I.R.	5.4 +/- 0.6	58.3 +/- 4.2

This data is in agreement with the observations on spontaneous mutation made by Muller (A1-15) in 1930 in which the role of background ionizing radiation in mutation in Drosophila was largely ruled out. Note that the largest effects on spontaneous mutation and gene conversion in yeast is determined by the repair pathways present and functioning.

Table A1-2 lists the effects of incubation temperature on spontaneous mutation and gene conversion. Even though the cell cycle time is longer at 22°C, and therefore the ionizing radiation background is greater, the back-mutation and gene conversion rates are

significantly lower than in the same strains incubated at 30°C. This indicates that the observed mutations and gene convertants arise from biologically catalyzed processes, which would be expected to show a strong temperature effect.

TableA1-2: Spontaneous gene conversion and mutation rates in yeast strains normal or defective for various DNA repair systems: incubation at 22°C. TRP+ = gene conversion; ILV+ = spontaneous mutation.

Strain:	Defect:	Radn. Sens:	TRP+/10^6 Cells	ILV+/10^7 Cells
Wild-type	None	Not	3.7 +/- 0.4	1.8 +/- 0.3
rad 3	UV-dimers	UV; not I.R.	8.0 +/- 0.8	2.8 +/- 0.3
rad 6	LCD	UV + I.R.	15.0 +/- 1.1	0.5 +/- 0.1
rad 52	DSB	I.R.	3.2 +/- 0.4	19.5 +/- 1.6

Just as in Tradescantia (A1-16), there is a marked reduction of spontaneous genetic events at a lower incubation temperature. The paramount effects of the repair systems in determining spontaneous levels of genetic events is obvious.

Invalidation of the doubling dose concept

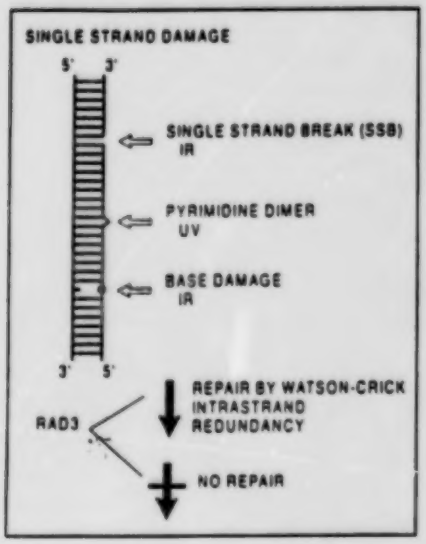
The data in these two tables invalidates the doubling dose concept, because the dose is minuscule over the ~24 hours of cell culture. By calculation based on the yields of radiation induced back-mutants and gene convertants from Figures 3 and 4 of the main text, less than one spontaneous back mutation or gene conversion in 50 to 400 thousand can be attributed to ionizing radiation background. And by observing 10-fold variations in spontaneous rates of either gene conversion (RAD 6 mutant strain) or back-mutation (RAD52 mutant strain) during normal growth for the same genetic changes, it is clear that biological variables control spontaneous mutation rates.

Exactly the same is expected of humans or other mammals. At about 10 uGy per day, a typical human cell with 6×10^9 base pairs will have about a 13% chance of a DSB or LCD per year from natural background. Billen (A1-17) reviews the rates of natural DNA damage events per cell, which are much higher than those attributable to ionizing radiation background.

Single strand damage repair - the RAD 3 system

By convention, mutations in a system are characterized by the corresponding RAD mutant number in yeast, in which these studies originated. The RAD 3 system deals with intrastrand damage such as UV-induced pyrimidine dimers (A1-18, A1-19). Mutants in

the RAD 3 system are not cross-sensitive to ionizing radiation damage. Our unpublished studies (Unrau et al) indicate that the yields of induced gene convertants and back-mutations in RAD3 mutant yeast strains are indistinguishable from the wild-type, and give no indication of cross-action of the RAD 3 system on ionizing radiation damage.



There is slight evidence that absence of the RAD3 repair system actually improves survival of yeast irradiated with ionizing radiation. This is unexpected, unless shunting all ionizing radiation damage down recombinational and post-replication repair systems leads to the repair of more lesions that are potentially lethal, than if some excision-repair is also active.

Repair of interstrand and coincident lesions - the RAD 6 and RAD 52 systems

The yeast RAD 6 and RAD 52 systems have marked effects on ionizing radiation sensitivity, and are thus implicated in ionizing radiation-induced DNA damage repair. It should be emphasized that these systems have their analogues in humans, mice and all living things. Modern genetic methods make the identification and mutation of genes analogous to the yeast genes easy in mice. From mice, extension to human genes and syndromes is straight-forward.

Mouse and human genes can be tested in yeast for their ability to compensate for yeast mutations at the functional level, by specifying the synthesis of repair proteins which form functional repair complexes of mixed human or mouse and yeast proteins.

The yeast RAD 6 system is thought to be active on DNA damage in which coding information is lost at replication. This would occur whenever, during pre-replication template checking, abnormal bases are removed, and the "empty" base locations repaired by the untemplated insertion of a DNA base. Evidence has been obtained (A1-20) that in

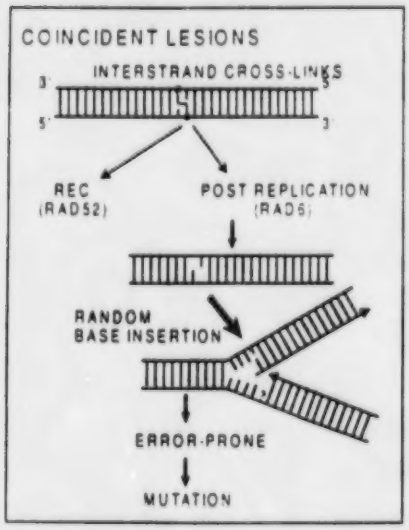
yeast the RAD 6 system involves protein complexes including a gap filling polymerase, DNA polymerase IV.

In Fanconi's anemia cell lines evidence has been obtained (A1-21, A1-22), implicating the Fanconi's system in the repair of DNA base gaps and inter-strand cross-links. The repair of interstrand cross-links leads to the involvement of the RAD6 pathway when chemically linked bases are excised without the breakdown of the phosphodiester DNA backbone.

In the main text, the sensitivity of the RAD 6 mutant strain to ionizing radiation is shown in Figure 2. The marked shoulder in the survival curve indicates that these strains retain the ability to repair some DNA lesions; these are expected to be DSB. Because the generation of mitotic gene-convertants is unimpaired in RAD 6 mutant strains, and because this is a recombinational end-point, the recombination repair of DSB must not be affected in yeast RAD 6 mutants.

Figure A1-3 is a cartoon of the general steps required for the repair of ISCL and/or LCD in DNA which is undergoing replication. Clearly an inter-strand cross-link will block DNA replication until it is removed. If it is removed by releasing the base from its sugar linkage, as is the case for alkylation damaged DNA bases (A1-23), leaving a gap in the replication template, then quasi-random base insertion by a gap-filling DNA polymerase will lead to mutation in some proportion of insertions.

The role of the RAD6 protein, which is a ubiquitin-kinase (A1-24), is then presumably to ubiquinate the polymerase IV-containing protein complex to ensure its degradation as soon as the gap-filling is completed, thus reducing the probability of further mutagenic DNA replication.



Tables A1-1 and A1-2 indicate that RAD6 mutant diploids have a very high spontaneous gene conversion rate, the implication being that ordinary metabolic activity always leads to spontaneous lesions of the sort normally dealt with by the RAD6 process. In the absence of RAD6-based repair, these spontaneous lesions are corrected by recombination with the homologous chromosome, leading to the hyperrecombinational phenotype.

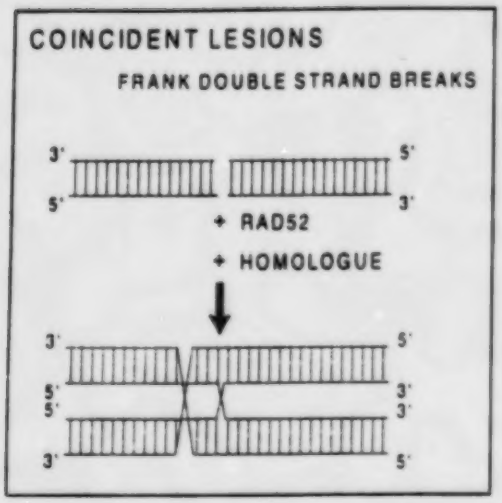
Thus, failure of the RAD 6 systems which repairs interstrand DNA cross-links and LCD leads to moderate radiation sensitivity. The ability to generate recombinational end-points in yeast and Tradescantia "pink" spots is however retained.

The yeast mutant strains most sensitive to ionizing radiation are those in the RAD 52 system. These strains are mutators under normal growth conditions, (Tables A1-1 and A1-2), and give indications that some new mutations can be induced by ionizing radiation. But there is no evidence of the induction of gene convertants, and meiotic recombination is also blocked. It is therefore concluded that RAD52 mutant strains lack the ability to repair ionizing-radiation induced DSB by recombinational processes such as those modeled in Appendix 2.

Note that there is spontaneous production of TRP+ clones in the RAD52 mutant strain (Tables A1-1 and A1-2), so that some form of genetic exchange is occurring within the TRP5 gene in this strain. Some elements of the recombinational repair system must still be intact, even in this recombination defective strain. The possibility that this recombinational capacity reflects non-homologous chromosome recombination, like the V(D)J system of generating immune variants in higher animals, is discussed in Appendix 3.

Figure A1-4 is a cartoon of the molecular events of recombinational repair of DSB. In essence, repair of DSB by recombination requires that they be transformed into single strand breaks (SSB) which can be repaired easily. Because DSB interrupt the phosphodiester backbones of both DNA strands, there are special problems associated with generating a pair of SSBs. The DNA sequence needs to be matched to that of a homologous DNA molecule, so that DNA base-pairing can be used to allow repair synthesis to occur. Models for the repair of DSB by recombination are based on Holliday's recombination model. Resnick (A1-12) originally proposed models for the repair of ionizing radiation-induced DSB and obtained evidence of repair failure in RAD52 mutant strains.

The salient features of recombinational DSB repair systems is that there is physical transfer of half a DNA molecule from one homologue to another, centered on a physical junction between the DNA molecules. That is, there is transfer of a Watson strand from one chromosome to pair with the Crick strand of the homologue, initiating a physical junction between the two chromosomes. The resolution of such Holliday junctions is discussed in more detail in Appendix 3. But it is the molecular resolution of these junctions which leads to the generation of some classes of LOH events, discussed in Appendix 4.



The other two ways of dealing with DSB are end rejoining, and non-homologous recombination repair. If DNA in chromosomes is organized in loops which are anchored at the nuclear cytoskeleton, then the DNA anchor points are intimately associated with topoisomerases which remove or insert superhelical turns into DNA molecules. A DSB in a loop could be rotationally translated so that both ends of the loop return to the nuclear cytoskeleton; if they wind up close together the concentration of DNA ends goes up to the point that they could become substrates for end-ligation.

As an alternative to homologous recombination repair the non-homologous recombination system which generates immune variants in mammals is implicated in the repair of DNA damage. This is evidenced by the ionizing radiation sensitivity of severe combined immune deficiency (SCID) mice (A1-25, A1-26).

Interestingly, there is evidence (A1-27) that the V(D)J non-homologous recombination pathway accounts for the repair of about 35% of genomic DSB. This implies that of the three repair systems presently implicated in the repair of DSB, namely homologous recombination, V(D)J non-homologous recombination, and end rejoining, work together to improve survival after ionizing radiation. The relative channeling of DSB into these three processes is unknown as is their specificity in the cell cycle, and for various types of DSB.

Non-homologous recombination repair uses either the ends of DNA molecules (without any base pairing), or limited structural homology such as is given by the immune system recombination signals, to pair DNA molecules for the repair of DSB. Clearly non-homologous recombination repair is a major repair pathway in the production of chromosomal translocations and deletions.

The somatic genetic and cancer risk implications of the various types of DNA lesions, the repair systems which act on them, and the genetic products of these repair systems are discussed in Appendix 3.

The random element in repair should be emphasized. A cell will never be able to determine which of two possible repair outcomes are without genetic consequence. The repair outcomes simply reflect the action of biochemical processes on DNA damage. The outcomes which can be scored are therefore simple reflections of the action of genetically determined repair systems.

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APPENDIX 2

ESTIMATION OF THE YIELDS OF DSB AND LCD IN IONIZING RADIATION DAMAGED DNA

Many direct estimates of the yields of ionizing radiation-induced DSB have been made (A2-1, A2-2, A2-3). Estimates for low LET radiations range from about 1 DSB per 1.25 x 10^8 base pairs at risk to around 1 DSB per 2.6 x 10^8 base pairs at risk. From the range in these estimates a yield of 1 DSB per 2.2 x 10^8 base pairs at risk per Gy is reasonable for purposes of calculation. Thus, in a human cell one might expect around 27 to 50 DSB per Gy, and for Tradescantia Clone 02, with its 6 x 10^{10} base pairs, between 250 and 500. This is unlikely to be in error by more than 100% for low LET radiations.

An estimate of radiation-induced LCD is much more indirect, and only one estimate is known to this author (A2-4). The basis of this estimate is extended and refined below. The conclusion is that LCD yields from low LET radiation are up to four times lower than from high LET and differ in both absolute yields and in oxygen cross section from yields of DSB. With high LET radiation, yields of LCD and DSB are not detectably different, though this may reflect an effect of lesion clustering from high LET radiation.

The theoretical basis of an estimate of the yield of LCD comes from studies measuring mitotic gene conversion in yeast strains which can or cannot repair LCD (i.e., which are sensitive to chemicals and radiations which induce LCD).

As argued in Appendix 1, the mutational inactivation of the RAD 6 process makes yeast strains sensitive to mitomycin C (MMC) and ethyl-methane sulphonate (EMS), in addition to ionizing radiation and UV. MMC causes LCD by linking G-G residues across the DNA double helix at CpG sites (A2-5), which are the preferred sites of cytosine methylation. When the interlinked G-residues are removed prior to replication (as they would block the migration of the replication fork), the gaps left in the DNA template will be filled by the gap filling DNA polymerase IV (A2-6). Figure A1-4 is a crude visualization of this concept. Yeast strains lacking the RAD6 repair process are both radiation-sensitive, and incapable of generating radiation induced back-mutations.

From the repair models in Appendix 1 it is argued below that the RAD 52 system, which functions in RAD 6 mutant yeast strains, converts both DSB and LCD into gene convertants, if they are in the gene conversion target. The dose response curves of gene conversion for both low and high LET radiations are linear for the RAD 6 mutant strain (as they are for wild-type), as shown in Figure 3 of the main text. Similarly, the yield of radiation-induced ILV1+ back-mutants in wild-type, (which can perform RAD6 dependent repair), is linear with dose, shown by Figure 4 in the main text.

The increased absolute yields of gene convertants in the RAD6 mutant reflects the processing of an additional amount of damage by recombinational repair, under the same mechanistic parameters as for DSB repair. Thus the additional gene convertants in the RAD 6 mutant strain, per Gy, relative to wild-type, reflect the addition of LCD-induced gene convertants to a number of DSB-induced gene convertants.

Table A2-1: Gene conversion in wild-type and RAD 6 mutant diploids as an indicator of the LCD induction rate: differences in gene conversion rates in the RAD 6 strain reflect recombinational repair of LCD + DSB by the functioning RAD 52 system; initial rates in wild-type reflect DSB repair only.

Strain:	Radiation:	N_2 Trp+/10^6/Gy	O_2 Trp+/10^6/Gy	Ratio DSB[a]
Wild-type	Low LET	2.6	7.1	1 : 2.7
RAD 6 mutant	Low LET	6.1	10.3	
Wild-type	High LET	6.9	11.9	1 : 1.7
RAD 6 mutant	High LET	13.4	23.8	

[a] The ratio of DSB is taken to reflect the increase in gene conversion yield between cells irradiated in N2 and in O2 with high or low LET radiation.

By subtracting the gene convertants which arise in the wild-type from those which are induced in the RAD 6 mutant, an estimate of the number of LCD hits in the gene-conversion target is obtained. Table A2-2 gives this value for anoxic and oxic, low and high LET radiation and calculations of the OERs and RBE for the induction of the two classes of lesions. The absolute yields of these lesions per base pair at risk depends upon the exact sizes of the various genetic targets the yields are derived from.

This data suggests that the relative yield of LCD on oxic low LET radiation is less, per base-pair at risk per Gy, than after anoxic irradiation. This implies a lower oxygen effect curve for such end-points as survival and gene conversion in the RAD 6 mutant strain than in wild-type.

Table A2-2: Yields, OERs, and RBEs of DSB and LCD calculated from the yeast data:

Radiation:	Lesions:	# in N_2	# in O_2	OER:
Low LET:	DSB	2.6/2.6= 1.0	7.1/2.6= 2.7	2.7
Low LET:	LCD	6.1-2.6/2.6= 1.3	10.3-7.1/2.6=1.2	0.9
High LET:	DSB	6.9/2.6= 2.7	11.9/2.6= 4.6	1.7
High LET:	LCD	13.4-6.9/2.6= 2.5	23.8-11.9/2.6= 4.6	1.8
RBE:	DSB/DSB:	6.9/2.6= 2.7	11.9/7.1= 1.7	
	LCD/LCD:	6.5/3.5= 1.9	11.9/3.2= 3.8[a]	

[a] The RBE for LCD yields based on these calculations shows the preferential induction of LCD by high LET but not low LET radiation. The possibility that densely ionizing tracks induce both DSB and LCD in the majority of events are suggested by this data.

On the assumption that no more DSB are induced in the RAD 6 mutant than in wild-type, and that the DNA target is the same size the yields of LCD per base pair at risk in the gene conversion target can be calculated. In Table A2-1 and A2-2 the relative yields of DSB and LCD are calculated from the increase in numbers of gene convertants per Gy in yeast RAD 6 mutant strains compared with wild-type. It can be seen that LCD are relatively more frequent per Gy than DSB in nitrogen than in oxygen, and have a lower O.E.R. than do DSB with low LET radiations.

In Table A2-3, the yields of gene convertants per DSB (and for RAD 6 , DSB plus LCD) in the target DNA is calculated, based on the expectation of 1 DSB per 2.2×10^8 base pairs at risk per Gy of low LET radiation. For 1 million cells, the gene conversion target is the 3600 base pairs in the TRP 5 gene within which a DSB (or an LCD) will lead to gene conversion by recombination.

The fact that yeast gene conversion experiments have been carried out to doses of 1 cGy suggests that the limits of this system have not been reached. Experiments to measure other genetic end-points in yeast will continue to be very cost-effective. One further area in which yeast experiments may prove cost effective is in the measurement of RBEs in larger and fully defined targets.

Table A2-3: Efficiency of gene-conversion induction by low and high LET radiation in the TRP 5 gene conversion target in yeast:

Strain:	DSB/3.6 x 10^9 B.pairs:	LCD/3.6 x 10^9 B. pairs:	Convertants/10^6 Cells:	%
Wild-type	16	7	7.1/16	44%
rad6	16	7	10.3/23	45%
Wild-type N.	27	27	11.9/27	44%
rad6 N.	27	27	23.8/54	44%

Column 4 gives the observed number of gene convertants relative to the calculated numbers of either DSB, or DSB + LCD in the total gene conversion target in 10^6 cells. The probability of a gene convertant per hit in the target is constant.

Unrau and Johnson (A2-7) suggest that about 50% of hits in the gene conversion target are expected to result in gene conversion if the induction of Holliday junctions is random with respect to the mutations in the gene undergoing conversion. 50% of lesions in the target would lead to gene conversion, and 50% would not. Within the limits of the accuracy of estimation of DSB induction, this calculation confirms the hypothesis.

The yields of convertants are within two-fold of the expectation of either DSB (or DSB + LCD in the RAD 6 case) physically induced in the gene-conversion target. These estimated yields are used in calculating the efficiency of generating DNA repair products including "pink" LOH events, in Tradescantia. The sizes of the various Tradescantia DNA targets are given in Appendix 3, calculated on the basis of the above relative yields of DSB and LCD, and corrected for a high probability of end-point generation for high LET radiation, and a low probability for low LET.

In light of exact target sizes arising from the complete sequencing of the yeast genome (A2-8) the production of mitotic recombination is about equally frequent in wild-type and in RAD6 yeast on the basis of lesions in the target as in Tradescantia Clone 02.

The suggestion is that one coincident lesion in the yeast gene conversion target, DSB for wild-type and DSB + LCD for the RAD 6 mutant, leads with 40 - 50% probability to gene conversion. But for a MCO target of 250,000 base pairs in wild-type yeast, only one lesion in 92 leads to MCO. This is similar to the proportion of lesions from low LET radiation which lead to MCO in wild-type strains of Tradescantia.

In other words, in yeast, 1 lesion ~ = 1 event for targets 3600 bp or smaller, and 1 lesion ~= 1/92 events for targets 250,000 base pairs or more. Losing the Clone 02 residual gene activity (in Clone 0106) means that about 1 low LET lesion in 20 leads to an MCO event.

Mutation target: From (A2-9) the number of mutations induced in the ILV1 gene and the rest of the yeast cell is, for 14.5 MeV neutrons, $4/10^7$ cells per Gy. From Table A2-2, there are 4.5/2.7 times as many LCD after high LET irradiation as after low LET irradiation. This implies a yield of 1 LCD/1.3×10^8 base pairs at risk in the yeast target for low LET radiation.

From strict target theory, if the LCD has to involve a specific base, the target is 52 base pairs. The ilv1-92 mutation is both suppressible and super-suppressible (A2-10) which means that there are at least three genetic targets. These are the original base changes in the ILV1 gene itself, and bases in the tRNAs and other genes which can mutate to recover the original ILV1+ enzyme activity. Certainly this target can be tested directly by DNA sequencing ILV1+ revertants to see if the structural gene itself has reverted.

For low LET irradiation in nitrogen, the calculation indicates that in a 52 base target, only 1 LCD would be expected in 4×10^8 base pairs at risk per Gy. The implicit assumption in these target calculations is that an LCD will be misrepaired with high efficiency to generate the end-points. There is much data from gene-conversion and back mutation in the CYC1 gene in yeast (A2-11, A2-12, A2-13) which can be interpreted on this model, using the same target calculations.

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APPENDIX 3

DNA TARGET CALCULATIONS FOR YEAST AND *TRADESCANTIA*

Coincident lesions present a special challenge to cells which must be overcome if they are to survive and leave offspring. In Appendix 1 the evidence for different kinds of coincident lesions and their repair systems was reviewed, and in Appendix 2 the yields of DSB and LCD with radiation dose was calculated. In this Appendix models of the repair intermediates and structures which are used to circumvent coincident lesions are presented, together with implications of such repair events for the generation of LOH events.

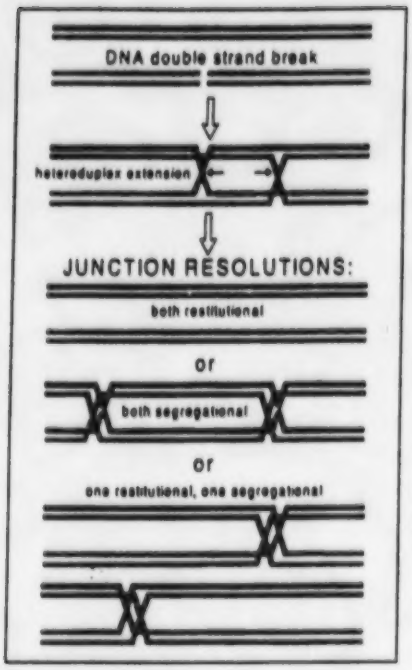
Mitomycin C (MMC) was found to be a recombinagen in wild-type yeast in 1964 (A3-1), so it is apparent that some part of MMC inter-strand cross links are repaired recombinationally. If so, this may represent a side reaction of the recombination systems which function in yeast and other organisms. It should be recalled from Table A1-1 that when the RAD 6 system for correcting LCD is inactivated, the rate of spontaneous recombination goes up about 10-fold.

Spontaneous LCD are therefore formed, detected, and repaired during normal cell growth. In the absence of the LCD repair pathway these lesions are repaired by recombination. Figure A3-1 is a cartoon of a recombinational repair system for the repair of radiation-induced DSBs, based on the recombination models of Holliday (A3-2), Resnick (A3-3), and Szostack et al (A3-4). The important feature is that the DSB is turned effectively into two single strand-breaks, by a process called excision-resynthesis.

Note that resynthesis always occurs in the 3' to 5' direction on a pre-existing template. So this means that excision-resynthesis proceeds in opposite directions from the newly formed single strand breaks. At some point in this process, the resynthesis stops when a stable linkage is produced in the strand which is being degraded by excision.

When resynthesis stops it leaves the two DNA molecules physically joined by the crossed DNA strands, at two places. Such Holliday junctions can by resolved by an enzyme called Holliday junction resolvase (A3-5, A3-6). As is apparent in the diagram, with two junctions there are two different outcomes of junction resolution. The junctions can be resolved "Restitutionally" ("R") or "Segregationally" ("S") at each junction (A3-7).

The importance of junction resolution is that if there is a gene between the recombination site and the end of the chromosome, and if the two homologous chromosomes are involved in recombinational DSB repair, then when there is an "S" resolution there is a good possibility that the gene will segregate in the next mitotic division. So if the cell survives but segregates a gene in one of its daughter cells, then that daughter cell and all its descendants will be genetically different from the mother cell and the rest of the somatic tissues.



That is, a new somatic genetic variant tissue may be generated by the continued division of the original recombined daughter cell. This in turn will lead to a patch of tissue in which repair, cell division, or other functions may be different from the parental tissue. It is this possibility of somatic genetic change in cells and their offspring which makes recombinational repair of ionizing radiation damage of concern in the cancer progression process.

Biochemically it has been shown that a Holliday junction has two-fold axis of symmetry and as a substrate can be cleaved equally likely in either plane. But this only holds true for a Holliday junction in open space free to rotate end-for end, and side for side. In the real physical world there are fewer degrees of freedom.

So while one might hypothesize a 50% probability that any given Holliday junction is resolved "R" or "S", in fact it is highly unlikely that the resolution probabilities will be equal. Holliday suggested that it would be found to be evolutionarily advantageous to generate largely "R" junction resolutions, so that LOH would be uncommon. This suggestion arose from the recognition that LOH of the dominant copies of recessive deleterious genes in heterozygotes would lead to somatic segregation and homozygosis of the recessive variants, exactly as is observed in LOH studies.

When a pair of Holliday junctions joining two chromosomes is considered, if the bulk of the chromosomes are undamaged, the junctions will join two huge polymers, and free

rotation in space is not expected. The involvement of helicases (A3-8) in DNA metabolism including the recombination process (and their implication in cancer risk in Bloom's syndrome (A3-9)) suggests some ways in which the physical interactions of chromosomes are dealt with. Helicases, for example, could introduce or remove biophysical torsional forces arising from the geometrical organization of the DNA, and from the biochemical events such as resynthesis occurring in a recombination event.

Reducing torsional forces would be expected to make junction resolution more equally "R" and "S". For two junctions close together, the expectation might approach 50% "R" and 50% "S", similar to expectations with freely rotateable model systems consisting of small recombination substrates.

The difference in "R" or "S" resolution probabilities for low LET recombination junctions and for high LET junctions between large DNA polymers may then lie in their spatial distribution. If you envisage recombination between two large polymers which have only a few DSB per chromosome, located far apart, then the local physical parameters of DNA may govern Holliday junction resolution probabilities. And local DNA organization may lead to independence of events in adjacent chromomeres.

For high LET repair, the numbers of LCD and of DSB are thought to be nearly equal (Appendix 2) and likely to be highly localized in the target of 2.6×10^8 base pairs (for Tradescantia "pink" LOH events) (See also ACRP-18 (3)). Furthermore, the absolute yields of DSB and LCD are two- to four-fold higher than for low LET. Taken together, I suggest that two or more coincident lesions and their repair events take place in the same target.

This means that any part of the DNA molecule which is included between two (double) junctions may well have more degrees of freedom for Holliday junction resolvase action than are available for a single junction. The hypothesis proposed to account for the large RBE of 0.43 MeV neutrons is that the odds of segregational resolution must be around 50%. This is expected to be far higher than for the resolution of independent and random recombinational intermediates from low LET irradiation.

This type of model suggests that the reason for dose rate effects with low LET radiation is that repair events start to change the probability of junction resolution when the lesions are close together in time and space to allow such interference. The time taken for resolution of Holliday junctions is typically several hours. Resnick and Moore (A3-10) showed that 50 % of DSB are repaired in CHO cells within 2.5 hours of irradiation. So doses which introduce new lesions into a repair domain while repair is going on have a chance of affecting repair outcomes.

The problem with this argument is that at a low enough dose rate of a low LET radiation, independent hits in the targets will not occur during a repair time. But the data in (1) and in (8) suggests that there is never a zero % chance of a DSB leading to LOH. Two alternative explanations are:

1. Because of the nature of ionizing radiation tracks, there is always a non-zero probability of getting more than one coincident lesion from the same track, no matter how sparsely ionizing. This is a matter for microdosimetric calculation. But such compound coincident lesions would be expected to alter the odds of junction resolution according to the above arguments.

2. There is always a non-zero probability that two junctions from a single DSB repair event will be resolved segregationally to generate LOH. This probability could depend on, for example, the concentration of helicases in recombination complexes. Then, if for example the helicases were rate limiting, as more repair events occur the probability of segregational restitution might increase.

Note that the cartoon shows recombination between two homologues, chromosomes which except for natural genetic variation are identical in sequence to each other. Indeed, there is good evidence that chromosomes in G2 (the cell cycle phase after DNA replication and before mitotic nuclear division) preferentially resolved DSBs by reference to the genetically identical sister-chromatids that result from DNA replication (A3-11, A3-12). But in G1 the most identical DNA copies available for recombination are the homologues, one donated by each parent. And there is genetic evidence in yeast (A3-13) that homologues are used to repair DNA DSBs.

There are two other recombination systems in cells which have evolved using non-homologous DNA molecules as a DNA repair substrate. One of these non-homologous recombination systems is used in the combinatorial generation of new immune antibody variants. The V(D)J recombination system does not require complete homology, but a simple nine-base sequence as a recombination initiation site signal. Any nine base sequence identical to this signal would be expected to be present about 23,000 times by chance alone in the whole human genome. Such a signal would break the human genome down into about 23,000 stretches of approximately 260,000 base pairs each.

If such a signal were the site from which non-homologous recombination repair initiates to the next such site, then DSB could be included in the recombined DNA region and deleted. But because the nine-base signal and its immediately contiguous bases served as the spot from which recombinational structures arise, most frequently recombination would appose non-homologous regions of the same or different chromosomes.

The normal V(D)J recombination which leads to the generation of antibody sequence variation normally excises the intervening DNA sequences as part of the resolution of the repair event. So the intrinsic segregational "S" resolution probability for this type of repair must be higher than for homologous recombination repair.

(The organization of the immunoglobin heavy chain gene is that 50 to 125 different V regions are available for recombination with the 4 to 10 J regions and the 20 - 25 C regions, within the same three million base pair region. Each successful antibody is a

combination of one of the V, J and C gene modules, subject to further cellular selection and somatic base pair mutation.

In the case of V(D)J recombination, enzymatically produced DSB must occur within a total gene complex of three million base pairs. In effect, V(D)J recombination always results from coincident "hits" within the same, relatively small target. And only the "S" outcomes can be processed to lead to potential antibodies. So in addition to coincidence of repair events, an alternative explanation for dose rate associated changes in the probabilities of "R" versus "S" resolution is that more of them are being repaired by non-homologous recombination, with an intrinsically higher probability of "S" resolution.)

Table A3-1 summarizes the data on production of various end-points in yeast as a function of the strains involved, and the numbers of initiating lesions per target. For survival, columns 5 and 6 give the numbers of DSB and LCD induced in the whole genome at the LD_{37} which is about one lethal hit per cell. For gene conversion, columns 5 and 6 summarize the numbers of lesions in the gene conversion target which give rise to gene conversion in the targets. Finally, unpublished data is used to calculate MCO per lesion in the last section of Table A3-1.

Table A3-1: DNA Targets and Calculated Yields of LCD and DSB in them:

Organism:	End-point:	Radiation:	Target Size (b.p.):	DSB/Target:[a]	LCD/Target:
Yeast w.t.	Survival	150 kVp	2.4×10^7 b.p./cell	~ 28	~ 9
Yeast rad 6:	Survival	"	2.4×10^7 b.p./cell	~ 12.5	~ 4
Yeast rad 52:	Survival	"	2.4×10^7 b.p./cell	~ 6	~1.8
Yeast rad 51:	Survival	"	2.4×10^7 b.p./cell	~ 2	~ 1
Yeast w.t.:	Gene Conv.	"	3.6×10^9 b.p./10^6 cells	~ 16 ~= 7.1 g.c.~7	
Yeast rad 6:	" "	"	3.6×10^9 b.p./10^6 cells	~ 16 + ~ 7 ~ = 10.3 g.c.	
Yeast w.t.:	Gene Conv.	14.5 MeV	3.6×10^9 b.p./10^6 cells	~ 27 ~ = 11.9 g.c. ~ 27	
Yeast rad 6:	" "	14.5 MeV	3.6×10^9 b.p./10^6 cells	~ 27 + ~ 27 = 23.8 g.c.	
Yeast w.t.:	MCO	150 kVp	3.2×10^{-5}/Gy/5×10^5b.p.[e]	~ 1 MCO / 92 lesions.	
Yeast rad 6:	MCO	"	3.2×10^{-5}/Gy/5×10^5b.p.	~ 1 MCO / 92 lesions.	

In Table A3-2 data from a number of Tradescantia studies are summarized in terms of the numbers of lesions required to either kill cells, or to generate "pink" stamen hairs. The numbers of "pink" events per Gy depends upon the dose, the dose rate, the LET of the radiation, and the repair competence of the Tradescantia clone.

Table A3-2 : Tradescantia targets and calculated yields of genetic events per the given number of DSB and LCD in them:

Strain:	End-point	Radiation:	Base-pairs:	DSB	LCD
Trad. 02	Survival	250 kVp	6×10^{10} b.p.	~ 614	+ 184
Trad. bloss.	Survival	"	4.3×10^{10} b.p.	~2220	+ 666
Trad. 02	"Pink"	250 kVp	2.6×10^{8} b.p. = ~ 0.06/hair/Gy	~ 6 /150	
Trad. 02	"Pink"	0.43 MeV [a]	2.6×10^{8} b.p = ~ 0.03/hair/cGy	~ 3 / 3.5	
Trad. hirs.	MCO	250 kVp	2.6×10^{8} b.p = ~ 0.01/hair/Gy	~1/150 [b]	
Trad. 0106[c]	"Pink"	"	2.6×10^{8} b.p = ~ 0.12/hair/Gy	~ 12/ 150	
Dose rate effects:					
Trad. 02	"Pink"	gamma	2.6×10^{8} b.p = ~0 03/hair/Gy	3 /150	
Trad. 02	"Pink"	gamma	2.6×10^{8} b.p = ~ 0.16/hair/Gy	16/150	

[a] Frankenberg et al (A3-15) estimate the yields of DSB from oxic alpha irradiation to be 2.5 fold higher than from 30 MeV electrons; Unrau (this review) estimates the combined yields of DSB and LCD to be 2.4 fold higher for oxic 14.5 MeV neutrons than for oxic 150 kVp x-rays. Therefore the expected lesions for 0.43 MeV neutrons should be adjusted upwards by at least these factors. Thus, the expectation is that 3 "Pink" events will be generated by about 3.85 "hits" in the target with neutrons; given the uncertainties, this seems a direct relationship.

[b] This 6-fold lower yield of events per Gy in Tradescantia hirsuticaulis, reported by Christiansen (21) than in Clone 02 may reflect the lower radiation sensitivity of wild-type strains to ionizing radiation, and a consequent lower yield of recombinants per lesion.

[c] Clone 0106 (4) was derived from Clone 02 by ionizing radiation and shows a significantly higher spontaneous "Pink" rate than others used in such studies. This would be expected if the gene for "spontaneous mutability", shown to be segregating in Clone 02 by the Mericles, had been itself subject to LOH by mitotic crossing-over, and was located on another chromosome than the color marker. The 16-fold higher spontaneous "Pink" rate of Clone 0106 than Clone 02 should be compared with the 8-11-fold higher spontaneous gene conversion rates observed in the RAD6 yeast strains compared to wild-type (Unrau and Morrison, in preparation; Appendix 2 Table A2-1). The 2-fold higher yield of "pink" spots per Gy for Clone 0106 falls within the range reported for the RAD6 strains after radiation.

Yields of translocations per Gy in human lymphocytes are linear with dose, and approximate one translocation per 1,000 coincident lesions. So it is very likely that non-homologous recombination is active in the repair of DSB. Indeed, it has been observed that SCID mice, which are knocked out in the ability to perform non-homologous repair, are sensitive to ionizing radiation (A3-16).

Again, if cycles of repair can occur (A3-17), the overall result would be greater radiation resistance of a repair competent wild-type. Non-homologous recombination is required for antibody diversity generation and carries a high risk of translocation and interstitial deletion. Homologous recombination carries with it the risk of LOH within a gene by gene conversion, and makes the target for LOH that span of chromosome between the risk gene and the centromere of the relevant chromosome. Cells can survive better after LOH by recombination than by translocation because all the genetic information is present, even if in only one of the two possible versions coded by the homologous chromosomes.

Target size calculations and assumptions

The yeast survival target is assumed to be the whole genome, as is the Tradescantia target. Thus the LD_{37}, which is that dose at which, on average, each cell has had one lethal hit, allows calculation of the numbers of lesions required to kill a cell. Of course, when on average each cell has had one lethal hit, some cells have not, and some have had more than one, but more than one lethal hit cannot be distinguished from one - dead is dead. The diploid yeast genome size is 2.4×10^7 base pairs; the Tradescantia Clone 02 genome size is ~ 6×10^{10} base pairs while T. blossfeldiana is about 4.2×10^{10} base pairs. Table 3 in the main text gives the LD_{37} for various yeast and Tradescantia strains.

The mitotic crossing-over target in yeast will be hit 92 times for each instance in which a mitotic cross-over is generated by wild-type. This suggests that the odds of "R" cleavage of Holliday junctions during lesion repair is 92-fold greater than of "S", or that only a subset of the lesions between ADE2 and the centromere can lead to repair events which generate mitotic crossing-over.

The mutant strains most sensitive to ionizing radiation are those in the RAD 52 system. These strains are mutators under normal growth conditions (Appendix 1, Table A1-1 and 2), and give indications that some new mutations can be induced by ionizing radiation. But there is no evidence of the induction of gene convertants, and meiotic recombination is also blocked. It is therefore concluded that RAD 52 mutant strains lack the ability to repair ionizing-radiation induced DSB by recombinational processes such as those modeled in Appendix 1 and 2.

TableA3-3: LD_{37} of Yeast repair defective mutant diploid strains:

Strain:	Repair Defect:	LD_{37}:	Shoulder:	OER:
Wild-type	None	260 Gy	Yes	2.5
rad 3	Excision	280 Gy	Yes	2.5
rad 6	LCD	115 Gy	Yes	1.8
rad 52	DSB	60 Gy	Yes	2.1
rad 51	DSB	20 Gy	No	N.D.

The point of this data is that, from RAD 6 and RAD 51/52 compared to wild-type, any single system knock-out leads to some but not complete radiation sensitivity, and that systems working in concert are more effective than any system working alone. Thus, the radiation resistance of the RAD 6 and the RAD 52 mutant strains implies that some residual DNA repair is taking place. But their sum is less than the radio-resistance of the wild-type which has both repair systems, and which can use them sequentially to enhance DNA repair.

Failure of the RAD 6 systems which repairs LCD and interstrand DNA cross-links leads to moderate radiation sensitivity but the ability to generate recombinational end-points such as gene convertants in yeast, and "pink" spots in Tradescantia is retained. This failure is linked to the absence of radiation-induced base pair mutations in the irradiated strains. Failure of the RAD 52 systems leads to extreme radiation sensitivity (to a few DSB) and to the absence of the induction of recombinational end-points. However, the induction of mutations indicates that these strains retain the ability to perform error-prone post-replication repair.

The somatic genetic and cancer risk implications of the various types of DNA lesions and their repair products are discussed in Appendix 5. The same mechanisms and repair systems are expected to perform the same functions, with the same somatic genetic implications, in all higher organisms.

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APPENDIX 4

MECHANISMS AND SIGNIFICANCE FOR CANCER RISK OF LOSS OF HETEROZYGOSITY (LOH)

Table 1 in the main text lists the seven most common molecular and chromosomal hallmarks for the loss of heterozygosity (LOH). The purpose of this appendix is to consider the general significance of LOH in the cancer process, and LOH as a surrogate for non-radiogenic and radiogenic cancer risk.

All cells, tissues, and organs in an organism are genetically determined, or programmed, and the limits to cellular growth and organization are genetically imposed. When cells grow out of control in a tissue or organ, they can form tumors, and these tumors may become life-threatening when they progress into cancers. In individuals who have no inherited mutations in any of the cellular, genetically determined somatic cell division control systems, a new mutation needs to occur in a critical gene, and this mutation needs to become homozygous (LOH) for a cell and its clonal descendants to grow into a tumor. This is the normal course of events for a tumor suppresser gene.

For oncogenes, the mutational course may consist of a coding change which makes the encoded protein more active than the normal. Alternatively there may be massive expansion of the numbers of copies of an oncogene, and/or of the encoded mRNA, in a gain of malfunction mutation. So LOH is largely concerned with loss of function changes such as the loss of tumor suppresser genes, while gain-of malfunction changes affect genes which stimulate growth in some way.

Most individuals already carry some three to seven deleterious recessive genes (A4-1) and in these individuals the initial mutations do not need to occur as they have occurred in the gametes of their ancestors. In these individuals, LOH in a somatic cell may lead to a different cancer risk than in a somatic cell without LOH. If the altered somatic cell escapes organismic surveillance and divides to produce a clone of altered cells, then these cells form the target for further genetic changes leading to cancer.

A good example is the case of Retinoblastoma, in which either an inherited mutation , or a new mutation arising during the formation of sperm or egg is present in a retinal cell (in which the normal gene copy is lost). This somatic LOH, by any of the mechanisms already demonstrated in Canavenee et all, (A4-2) leads to tumors of the retina.

Rb- LOH usually arises during gestation, and thus does not reflect environmental causation, but pure somatic expression of a genetic cancer risk factor. This cancer risk depends upon LOH of the Rb+ gene. The actual somatic genetic situation is therefore, Rb+/Rb- → Rb-/Rb- → eye tumors →metastases in the brain → death.

Thus, Rb is a fairly simple case conforming to Knudsen's multi-step carcinogenesis model (A4-3), with only a few steps in the progression process. This may be simpler than adult onset cancers, but it does speak to the central problem of LOH in carcinogenesis. It is expected that in more complex cancer types, a more extended sequence of somatic genetic changes may be needed to lead to the development of full-blown cancers. But a process of sequential loss of cell division control genes in individual cells and their clones, leading ultimately to loss of cell division control in a cancer, fits the data well.

Now, the relationship of LOH to cancer risk is far broader than its derivation from one specific cancer type. In a recent survey of the references in MEDLINE to LOH and cancer progression, more than 700 citations to LOH and its' significance in particular cancer types was found. Twenty examples from this survey are given in Table MT-5 in which the loss of specific parts of chromosomes, or genes, or even of mutation within genes (which also lead to LOH) was observed. As the application of molecular analysis techniques to tumors and cancers evolves, LOH properties will be diagnostic of particular cancer types.

Table A4-1 recapitulates the target sizes for the sorts of somatic genetic changes of interest in determining ionizing radiation-induced cancer risk.

Table A4-1 LOH target Sizes in Yeast and Tradescantia:

Class:	Target Size in Base Pairs:	Probability of Event/Lesion:
1 Mutation	~ 50 - 5000 b.p. /gene	Unknown
2 Mit. gene conv.	3,600 b.p. trp5-12/27	~50%
3 Mit. cross-over	249,000 b.p.	~1-3%
4 MCO "pink"	2.6×10^8 b.p. Clone 02	100% neutrons; 2% 250 kVp
5 Deletion	Parts of a gene	Unknown
6 Chrom. Arm Loss	Including gene	Colorless/Dwarf in Clone 02?
7 Chrom. Loss	Deletion	?

The relevant questions for radiation protection are:

1. Is there any particular mechanism of LOH more associated with ionizing radiation damage than from spontaneous damage? Is there an ionizing-radiation specific LOH signature?

2. Are there significant numbers of individuals in ARW or other populations who are at enhanced cancer risk because of LOH induced by ionizing radiation at low doses and dose rates?

3. Because the evidence for linearity in LOH induction by ionizing radiation in plants, yeasts, and other organisms seems adequate to accept the working hypothesis, the major uncertainty about linearity is the extent to which it occurs in actual human individuals.

Appendix 3 discussed the resolution of DSB repair intermediates to generate LOH events.

But from the data in Tables A3-1 and A3-2, Appendix 3, which summarizes all the information about DNA lesions and the generation of LOH end-points in yeast and in Tradescantia, not all DSB and LCD lesions repaired by recombination lead to long range LOH, especially not low LET lesions. For wild-type yeast, in the 249,000 base pair ADE 2 mitotic cross-over target, only ~1 lesion in 100 leads to MCO.

In Tradescantia Clone 02 and 0106 respectively ~ 1/40 or 1/20 of low LET lesions in the target lead to the "pink" mitotic cross-over. In T. subacaulis, the probability of a "pink" is about 1 lesion in 100, and in T. hirsuticaulis the probability is only about 1 lesion in 200. So for low dose, low dose rate low LET radiation, the information from both yeast and Tradescantia wild-types is that there is a low probability per repair event of a recombinational repair junction leading to a MCO event when it is resolved.

Continuing with this argument, for most of the end-points scored, in both Tradescantia Clone 02 and in T. subacaulis , irradiation with higher dose rates and doses of low LET radiation increases the probability that a DNA lesion will be resolved to generate an MCO event. This is shown by the positive slopes for the generation of the various MCO classes with doses of 250 kVp X-rays between about 5 and 100 cGy in the main text, Figure 1b. But the absolute levels of yield never intersects the high LET curves, and the slopes never reach +2 which would be expected if two "hits" are always needed to generate the end-points. In fact, the observed slopes of ~+1.2 - +1.4 indicate a "J" curve.

Note that the lower part of this curve is linear with a slope of +1 for Clone 02, and that there is no sign of a threshold below which there are no "pink" events. The linear part of the curve implies that there is a constant probability that the recombinational junctions will be resolved to generate a "pink" MCO, in the repair of a coincident lesion. This probability is somewhere between 1/100 and 1/50, irrespective of the dose. This lack of dose and dose rate effect implies that a biochemical minor product, namely an "R" - "S" resolution, occurs at the lowest doses and dose rates of low LET radiation.

The sizes of the radiation targets are listed in Table A4-1 in increasing size, taking for example both yeast and the Tradescantia systems. In the further discussion we can leave out direct radiation-induced base pair changes (mutations). As implied above, recombinational targets are much larger. In Table A4-1 the gene conversion target is estimated at about 3600 base pairs; for large yeast populations this makes an experimental system which can easily be manipulated.
The mitotic crossing-over target is much bigger than the gene conversion target, because any recombination event initiated between the chromosomal centromere and the gene may lead with some probability to LOH. From Tables A3-1 and A3-2, this probability is about 1/100 for low LET lesions in the target in wild-types.

This implies that "S" events after low LET radiation are much less probable than after high LET lesions in this and in other systems which show a high RBE. But the RBE of neutrons has not been measured in both the wild-type and RAD6 mutant yeast strains for MCO; an increased RBE in RAD6 would implicate recombination repair mechanisms in the generation of MCO.

Finally there are the targets which reflect outright loss of chromosomal DNA by chromosome breakage without reunion, which range from parts of chromosome arms to whole chromosomes. In yeast, mitotic non-disjunction scored by measuring clones which have lost a chromosome is a readily saturated process, with low dose linearity of kinetics followed by a decline in the number of events at higher doses (A4-4,A4-5).

No evidence is reviewed about this process in higher organisms. However the Mericles (A4-6) have correlated the occurrence of micronuclei with the generation of various types of cell aberrations in Tradescantia and have shown that chromosome loss is not associated with the generation of mitotic crossing-over events.

The conclusions of this appendix are therefore that the actual DNA targets which are measured vary in size from tens of base pairs for mutation to thousands for gene conversion, and to many millions for mitotic crossing over.

Linear non-threshold dose response curves are expected in humans who are also partially or wholly deficient in the RAD 6 pathway, such as Fanconi's anemia gene carriers. LOH of a copy of the normal Fanconi's anemia gene in a somatic cell could give rise to somatic cell clones in which cancer probabilities were much higher than in the normal body cells (A4-7). The observation that Fanconi's anemia homozygous cell lines are hyperrecombinational suggests that clones in which LOH had occurred would also be hyperrecombinational. Recombination as such would lead with increased probability to further steps in the multi-step cancer progression process. This is a reasonable expectation given the clonal origins of cancers.

Finally the results in Tradescantia and yeast are relevant to consideration of the expectations of radiation induced LOH in humans. Hundreds of references to LOH in

human cancers indicate that if ionizing radiation is an especially efficient inducer of LOH, it may, in some individuals serve as a special cancer risk source.

Excess sensitivity of cells from obligate Ataxia telangiectasia heterozygotes (A4-8) to low dose, low dose rate, low LET radiation has been observed. This makes the 0.5 to 1.5% of AT heterozygotes in the human population a group of special concern for radiogenic cancer risk arising from AT-gene LOH events. It should be pointed out that this germinal study has been extended to include cancer patients which are hypersensitive to ionizing radiation (A4-9) with results indicating that some of them form a radiosensitive population subgroup.

This biological risk factor should be taken into account to protect At heterozygotes to the same extent as is afforded to non-heterozygotes for cancer risk genes.

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APPENDIX 5

LINEAR DOSE RESPONSES FOR GENE CONVERSION IN WILD-TYPE AND RAD 6 MUTANT YEAST AND MITOTIC CROSSING-OVER INDUCTION IN *TRADESCANTIA* SPECIES

Data from gene conversion studies in wild-type yeast strains has been discussed in the context of observations relevant to the linear - non threshold debate [ACRP-18 (3)]. This data is supplemented with data from the mutant RAD 6 yeast strain, obtained under the identical irradiation conditions to make two points: the yield of gene convertants is linearly related to dose in both cases, and the absolute yield of gene convertants per Gy is twice for the mutant RAD 6 strain what it is for wild-type, reflecting the repair by gene conversion of lesions normally repaired by the RAD 6 "error-prone repair" system.

In Figure 3 the yields (less spontaneous initial convertants) of ionizing radiation-induced gene convertants are log-log plotted for convenience of scale. For wild-type, over a range of 2.5 to 25 cGy of 14.5 MeV neutrons the dose response curve is linear, and for 150 kVp X-rays (plotted from Unrau, Zimmermann and Johnson (A5-1)) with the relevant comparison experiments from this series overlaid, there is clear linearity of end-point production down to 1 cGy and up to 50 Gy. 150 kVp X-rays give an oxygen enhancement ration of 2.7 for the wild-type. The gene conversion data for wild-type comparing 14.5 MeV neutrons with 150 kVp X-rays is consistent with an RBE of 1.7, and an oxygen enhancement ratio for the neutrons of 1.7.

The RBE of 1.7 is expected on microdosimetric grounds if the yield of the relevant recombination inducing DNA lesions is 1.7 times greater for 14.5 MeV neutrons than for low LET radiations.

Also shown on Figure 3 is a plot of the gene conversion data for the RAD 6 mutant, on the same dose response curve as wild-type. Again, yields over the range 6.25 to 150 cGy give linear dose responses for both 14.5 MeV neutrons and for 150 kVp X-rays. For the RAD 6 mutant the OER for X-rays is 1.7, and for 14.5 MeV neutrons is 1.8; the difference in OER between wild-type and the RAD 6 mutant for X-rays is significant, while that for neutrons is not. What is apparent in Figure 3 is that the yield of gene convertants per Gy for the RAD 6 mutant is 1.7 times higher for low LET radiation, and two times higher for high LET radiation, than wild-type.

It appears that while the repair deficiency in the RAD 6 mutant strain increases the numbers of gene convertants/Gy, and changes the OER to reflect the presence in the repair pool of lesions with a differing radiation induction cross section, the dose response curves for the generation of the end-point are still linear to the lowest tested doses. At these doses we expect only single DSB or LCD to be induced in the target DNA of cells which undergo gene conversion.

Tables A2-1 and A2-2 in Appendix 2 gives the detailed analysis of the yields of gene convertants as a function of gassing conditions and cell strain which were used to derive the yields of DSB and LCD in the target gene in yeast. The above data are shown in Figure 3 to confirm that there are linear dose response curves for both the wild-type strain and the RAD 6 mutant strain, and that the yields of convertants per Gy are consistently higher in the RAD 6 mutant strain.

Clone 02 and its more radiation sensitive derivative Clone 0106 yield at least 10 X more events per Gy than T. hirsuticaulis; five-fold more than T. subacaulis, and Clone 0106 yields close to twice as many events per Gy as Clone 02 (A5-2, A5-3, A5-4).

Figure 2 shows data from 0.43 MeV neutron-irradiated Tradescantia Clone 02 and T. subacaulis. Figure 2a shows that the remarkable slope of yield versus dose for the "pink" end-point for Clone 02 is linear with a slope of +1.0. There is no dose rate effect over three orders of magnitude of dose rate (1). This means that the end-points are induced directly proportional to high LET dose; one lesion in the operational target approximately one end-point.

But for some endpoints induced by neutrons in some strains the slopes are less than +1.0. This means that not all lesions in the operational targets lead to the end points. Several testable alternative interpretations of this data can be derived. From the yeast gene conversion data in Appendix 2 it appears that the absolute probabilities of DSB and LCD are near 1 for 14.5 MeV neutrons. Myers and Johnson (3) have argued that on microdosimetric grounds high LET radiation should induce clusters of lesions because of the dense ionization in their track.

This means that, at the DNA level, one can visualize clusters of lesions making up random mixtures of DSB, LCD, and other types of DNA damage. Such a cluster, in the large target implied by the yields of "pink" hairs in Clone 02, must have a very high probability of being repaired in such a way as to generate a mitotic cross-over. But these clusters themselves will have varying micro-structure with respect to the lesions and their spatial arrangement.

Assuming that only two lesions were present in a cluster, some combination of DSB and LCD, then a binomial distribution of lesion pairs is expected. For pairs of LCD in a cluster there is no necessity of recombinational repair if the RAD 6 pathway is operative during DNA replication; the lesions are coincident but the DNA backbone is not broken.

Similarly, for a pair of DSB in a cluster, two recombinational repair events need to occur within the mean distance apart of the pair of DSB. This could easily result in the loss of the intervening DNA fragment, depending upon the actual distance apart of the DSB in base pairs. But if repair of both DSB does proceed, then their proximity may greatly increase the probability of segregational Holliday junction resolution.

Finally, ½ of the lesion clusters are expected to be mixed lesion clusters, which have both a DSB and an LCD lesion within a short distance in base pairs. If the RAD 6 process is functioning properly, this class of lesions would be repaired by the presumably sequential action of the RAD 52 recombinational process, and the RAD 6 LCD repair process.

So unless the sequential repair changes the probability of segregational Holliday junction resolution, not all such lesions would give rise to the "pink" mitotic cross-over end-point. But if Clone 02 is partially defective in the RAD 6 process, then the probability of making a recombinational end-point in Clone 02 would be higher than in wild-type Tradescantia.

It is a combination of the microdosimetric distribution of two major types of DNA lesions, and the repair interactions of the two major repair pathways which have evolved to deal with these lesions, which lead to the biological end-points being scored. In no case is the end-point not produced; the slopes observed merely reflect the probabilities that lesions will be processed to generate end-points or not.

On this argument, pairs of LCD in a mutant RAD 6 strain have to be resolved by recombination when they are detected by their interference in the DNA replication process. It has long been known that blocking DNA replication as a process leads to the induction of recombination (A5-5).

The recombinagenic nature of UV, ionizing radiation, and many other agents can be attributed to a block in DNA replication inducing recombination. Indeed, meiosis, the process evolved for recombination, is a case in which two cell divisions follow a single round of DNA replication; it may be that the relative block in DNA replication is the general trigger for meiotic recombination.

The linearity of dose responses of Clone 02 and the segregation both in meiosis (A5-6), and (after irradiation (A5-3)) in mitosis of a gene controlling radiation sensitivity in its offspring and sub-clones, indicates that Clone 02 is heterozygous for, or is a "carrier" of a gene which affects radiation sensitivity, spontaneous LOH rates, and the radiation induction of LOH events.

This gene is hypothesized to be the Tradescantia equivalent of a gene in the RAD 6 pathway of yeast. The data summarized in Figure2a supports this contention, by indicating that there are more LOH events in Clone 02 than in the alternative species, and that in Figure 2b the Clone 02 sub-clone, 0106 is even more radiogenic for the LOH end-point and has a higher spontaneous mutation rate than Clone 02.

Sparrow and Sparrow (A5-7) have reviewed spontaneous "pink" event rates in various Tradescantia species clones. Rates vary between $0.69/10^3$ hairs for Clone 02 and $17.65/10^3$ hairs for Clone 0106, 18 fold-higher. A further range from $2.15/10^3$ hairs for T. subacaulis #4405 to $16.98/10^3$ hairs for T. subacaulis #4463 is also observed. The overall range of spontaneous "pink event' frequencies is thus about 20 fold for

Tradescantia clones of various sorts. Christianson (A5-4) measuring mitotic crossing-over had a spontaneous rate in his T. hirsuticaulis clone of $1.35/10^3$ hairs, a low rate of somatic genetic change. The range of spontaneous "pink" genetic event frequencies is as large as is observed in the various yeast DNA repair strains summarized in Appendix 1, Tables A1-1 and A1-2.

LOH in Other Plant Systems

In heterozygous tobacco plants LOH is scored in leaf cells by observing the dose-related induction of green cell clusters on a yellowish green background. A number of studies of gene conversion and chromosomal non-reciprocal reversion have been published (A5-8, A5-9, A5-10). In the very low dose rate study of Fabries and Delpoux (A5-11) the slope of yield is not significantly different than +1 at dose rates between 0.16 and 0.04 mGy/min; the data is presented in Figure 8. In the intermediate dose and dose rate study of Dulieu and Dalebroux (A5-10) the slope of recombinant yield against dose is greater than +1, and less than +2, as would be expected at the dose rate used, 6 Gy/min, based on studies of dose rate effects on Clone 02.

The central argument in this Appendix is that, while Clone 02 is partially defective in the repair of LCD, and this partial deficiency is associated with greater yields of MCO events per lesion of high or low LET radiation, there is no evidence whatever to suggest that wild-type Tradescantia species do not recombine after ionizing radiation to generate LOH end-points. This means that in humans, high LET radiations are expected to generate LOH events to the very lowest doses and dose rates.

The Tradescantia data for low LET radiation suggests that the lesions induced differ in spatial distribution, absolute yields, and relative proportions of DSB and LCD. The upward inflection of the dose response curves summarized in Figure 2a and 2b above 2 to 10 cGy at dose rates above 5 cGy/min suggest that lesions which accumulatewithin a repair interaction distance of each other change the probability of LOH. For low LET lesions there is a low probability that segregational outcomes will result from repair, and this probability depends in part upon the density of lesions in the effective targets.

But there is never a slope of +2.0 which would be expected if two lesions of any type in the same target invariably lead to a segregation event. This opens the possibility that only combinations of lesions, such as, for example pairs of DSB, or combinations of one DSB and one LCD, within the same repair target, increase the probability of segregational Holliday junction resolution.

From Appendix 2 it was calculated that aerobic DSB were twice as common as LCD after low LET radiation. Of possible pairs of lesions, about 40% would be pairs of DSB; 50 % mixed lesion pairs, and 10% pairs of LCD. The observed positive slopes in Tradescantia after low LET radiation, if they reflect interaction between repair events to alter the probability of segregational outcomes, suggest that pairs of DSB are most likely to trigger this effect.

Once again, neither microdosimetric or repair considerations in isolation are adequate to explain the data; both need to be considered. From the data it is clear that even low LET radiations at low dose rates have a finite probability of inducing MCO or other LOH end-points. This probability never drops to zero in any system examined. So the lesion and repair dependent basis of the linear non-threshold interpretation seems valid.
From Figures 2 a and 2 b it is clear that the yield of low dose low LET end-points is offset from the high LET curves by about 50- to 100-fold. This is no evidence of a threshold, but an indication of the relative efficiency of the repair of lesion clusters after high LET radiation at generating LOH.

In yeast, the relative efficiency of generating gene conversion is about 1.7-fold bigger for 14.5 MeV neutrons than for 150 kVp x-rays. The probability of coincident hits in the 3600 base pair gene conversion target is negligible, so there is no possibility of repair events within the target domain interacting.

Explanations based on both microdosimetry and repair thus have the potential to integrate the physical and biological effects of ionizing radiation in an understandable way. For example, the observation that RBE depends upon target size in base pairs (ACRP-11; ACRP-18) can be explained by a combination of the types of lesions involved (DSB versus LCD); the types of repair processes involved (RAD 52 recombination versus RAD 6 error-prone repair and a combination of both RAD 52 and RAD 6 repair); the types of LOH produced (base pair sequence changes versus DNA exchange), and the dependence of the LOH change on the molecular events at the resolution of Holliday junctions, and their spatial and temporal distribution in the target.

Thus for systems in which small RBEs are observed it is expected that the target is small, reflects the repair of lesions in or around that target, and is a highly efficient monitor of lesions and their repair within that target. But in systems with high RBEs, the expectation is that the efficiency of repair of low LET radiation lesions to generate the end-point is low, and depends additionally on the spatial and temporal distribution of lesion within the target.

The further expectation is that high LET lesions in big targets also have a high probability of generating the end points being scored. These expectations can be tested by reference to the relevant literature and by the experiments suggested in the main text to test LOH and cancer risk in mice.

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APPENDIX 6

LESION INTERACTIONS, REPAIR DOMAINS, AND LOW VERSUS HIGH LET EVENTS IN *TRADESCANTIA* SPECIES

Ichikawa, et al. (A6-1) report dose rate effects of low LET gamma radiation on the killing of stamen hairs in Tradescantia Clone 02 in comparison with a wild-type species, Tradescantia blossfeldiana. Based on LD_{37} values obtained at high dose rates (0.293 Gy/min for Clone 020, and 2.56 Gy/min for T. blossfeldiana,), versus intermediate dose rates 1/1000 to 1/60th for Clone 02, and 1/500 to 1/60th for T. blossfeldiana, it was found that both species showed a marked dose rate effect for survival, of about two in either case.

Table 1 gives the LD_{37} doses obtained by inspection of the data; the numbers of DSB and LCD expected to be induced in the large DNA-rich cells, the dose rate effect values, and the relative sensitivities of Clone 02 compared with T. blossfeldiana.

Table 1: Effect of low LET dose rate on cell lethality in Clone 02 compared with a wild-type Tradescantia species, and relative radiation sensitivity of Clone 02 on the basis of lesions per base pair at risk.

Strain:	Bp/genome:	LD_{37} :	Lesions:	High D.R.	Low D.R.	Dose Rate Effect:
02	6×10^{10}	2.3/4.2	DSB	630	1150	1.8
			LCD	190	345	1.8
T. bloss.	4.2×10^{10}	11.5/26.0	DSB	2195	4960	2.3
			LCD	600	1500	2.3
Relative Sens. : 02 / T. blossfeld.				3.5	4.5	

The two clones can be compared for sensitivity on total lesion per genome basis. Clone 02 is absolutely more radiation sensitive and has a lesser dose rate effect than T. blossfeldiana. This is expected if Clone 02 is defective or partially defective in the RAD 6 repair process. The dose rate effects suggest that interaction of repair events may account for part of the increased sensitivity at higher dose rates. That is, T. blossfeldiana has both a greater dose rate effect as such, and an incremental increase in radio-resistance, as a function of lower dose rate.

Now, for the RAD 6 yeast mutant, the LD_{37} obtained in oxygen is about 2.3-fold smaller than that of the wild-type, and in nitrogen about 3.7-fold, and these effects of the absence

of the RAD 6 repair system, which really reflect the greater radiation sensitivity per base pair at risk, are of the same order as the relative sensitivities of the two different Tradescantia species. The hypothesis is that Clone 02 is mutant in the Tradescantia RAD 6 pathway.

Dose rate effects are expected if Clone 02 has decreased activity of a repair system which acts later in the cell cycle than the major DSB repair system. A RAD6 system, acting as it does during replication, would lead to greater radiation sensitivity if more lesions need to be repair by recombinational means, prior to replication.

The effects on DDREF of incubating Clone 02 at lower temperatures (A6-2), indicates that lesions "pile up" before cell division at the lower temperature and are effectively coincident with respect to cell division. More lesions accumulate at the lower temperature, giving a greater effective dose rate, and thus higher probabilities of coincident lesions per base pair at risk. The resulting repair competition leads to higher probabilities of generating "pink" events per lesion.

It should be emphasized that Tradescantia plants normally grow in temperate conditions, and are adapted for optimal repair near room temperature. Mammals, however, are adapted for homiothermy and optimal repair at 37°C. So when plants are dropped 12°C from 20°C to 8°C, the effects on slowing down DNA repair are similar to dropping animal cells from their normal 37°C to 23°C, as happens when irradiations are performed in animal cells at room temperature. So the added effects of lower temperature irradiation are that lesions coincide in repair time at the lower temperature which would not do so at normal incubation temeratures.

The implication is that for low LET lesions, there is a coincidence factor which is related to the concentration of lesions in time to observe dose rate effects. Is there further evidence that lesions must coincide in space, for more efficient end-point generation?

Repair of high LET lesions generate end-points far more efficiently than low LET lesions in Tradescantia, as demonstrated in Appendix 3. But from Appendix 2, Table A2-1, it is apparent that yeast very efficiently repair both LCD and DSB within the gene conversion target, from both high and low LET radiations, to generated gene convertants.

However, because at higher dose rates of low LET Clone 02 generates more "pink" end-points, the probability of the end-point per DNA lesion, must go up as a function of the number of lesions in the same target.

The argument is as follows: High LET radiations always generate compound coincident lesions because of their high ionization density. The evidence from Appendix 2 that the numbers of lesions, and the relative proportions, are near unity for DSB and LCD with high LET radiation in yeast is transposed to Tradescantia. The expectation is that high LET lesions will always coincide in time, and either be in the target or not. When they

are, judging by the calculations in Appendix 2, they are very likely to generate a LOH event.

Low LET lesions will coincide in time and space only on a Poissonian probability basis, depending upon the target size, the rate of lesion input, and the rate of lesion removal for their coincidence.

The probability of two low LET lesions coinciding must then depend upon the size of the target in base pairs, the dose rate, and the repair rate for coincident lesions of both types. Coincidence will increase as any of these factors increase; this is evidenced by the dose-rate dependence of slope of "pink" events in Reference 1 at the lowest doses and dose rates. In Reference 1, the slope of the curve of "pink" induction at dose rates below 0.52 cGy/min is not distinguishable from +1.0, while at 0.293 Gy/min the slope is +1.24. Other slopes for somatic genetic events in T. subacaulis, range from +1.17 to +1.70 with high dose rate low LET radiation.

For small targets of the order of a few or a few thousand base pairs (e.g. mutation and gene conversion in yeast) the probability of lesions coinciding in the target is effectively zero. So the efficiency of generating end points must depend only on the cross-sectional yields of the various coincident lesions, the probability that they are within the base pairs of the target, and the probability that a repair event will generate and end point.

For mutation this has been calculated at a few % (Unrau, 6-3). For gene conversion on both a theoretical (Holliday, A6-4) and an observational basis, the probability is 50% for a lesion repair event in the target generating a gene convertant. This is consistent with the calculations presented in Table A2-3, Appendix 2.

Christiansen (A6-5) showed that in T. hirsuticaulis the "pink" end-point represents a mitotic cross-over. Assuming that this is true in Clone 02, the target can then be defined as those base pairs in the chromosome on which the "pink" and "blue" markers are located, between the "pink" gene and the center of the chromosome. This is because a mitotic cross-over has to take place to permit the segregation of the "pink" gene into only one cell nucleus after cell division.

The probability of a few % after low LET radiation implies that the lack of coincidence of lesions in the same space and time leads to the low probability of generating an event. As the dose rate goes up, coincidence increases. This has been interpreted to imply that two lesions are always required, leading to a dose squared expectation. But none of the data reported for any of the Tradescantia strains, end-points or radiations attains a slope greater than +1.7, and the bulk of the slopes reported reflect a slope of around +1.4 (MT Table 6).

As argued in Appendices 1 and 2, there are two classes of coincident lesions, and after low LET aerobic radiation pairs of such lesions in the same repair domain would be

expected to be about 50% pairs of DSB; 40% mixtures of 1 DSB plus 1 LCD, and 10% pairs of LCD.

The probability of two independent and different lesions at the lowest doses and dose rates of low LET radiation for one ionizing radiation track may not be 0. Then there would always be a number of such lesions with a higher probability of being repaired and generating the "pink" end-point. A Poissonian density function might describe such lesions in the large "pink" target.

For Clone 02, after high LET radiation, the probability of this segregation is nearly 100%, based on the number of "hits" expected in a chromosome arm. For high LET radiations the probabilities would be that 25% of pairs of lesions would be DSB; 50% mixed, and 25% LCD.

From dose response curves we can compare the efficiency of Clone 02 with T. subacaulis. The Clone 02 slope is not different than +1.0, while the T. subacaulis slope is significantly less than +1.0, at +0.75. It is a reasonable deduction that classes of damage which in Clone 02 lead on repair with high efficiency to "pink", are repaired in some other way in T. subacaulis.

The most direct interpretation is that in T. subacaulis pairs of LCD are repaired by error-prone repair and do not lead to the end-point, while they are repaired by RAD 52-catalysed recombinational repair in Clone 02 and do lead to the "pink" end-point. This is consistent with the straight line dose response curves, as fewer than 100% of lesions in T. subacaulis ever lead to the end points, while all of them may in Clone 02.

A repair domain can be defined as the length of chromosome arm within which two compound coincident lesions lead with high probability to the end point. For high LET, the mitotic crossing-over target is calculated at 2.6×10^8 base pairs with about a two-fold range of size depending upon exact lesion yields. From Appendix 3, the probability of a lesion in that target, and its repair to generate a "pink" is close to 1.

In this analysis, it is assumed that the same target size for "pink" end-points is found in all the various clones and strains in which the end-point is scored.

If the same target size is used to calculate coincidence of low LET hits, then at varying dose rates as few as three lesions in 150 in that target lead to a "pink", and a maximum of 16/150 may. Depending on lesion coincidence in space and time, there is a six-fold difference in the probability that a low LET lesion, or a pair of lesions, will lead to an end-point.

Five factors affect "pink" yields: the absolute density of lesions; the absolute probability of compound coincident lesions from individual ionization tracks as such; the coincidence of lesions in time: the coincidence of lesions in space; and the chemical nature of the coinciding lesions (i.e. DSB/DSB, DSB/LCD, or LCD/LCD). In addition

to these micro-dosimetric factors the data reviewed from yeast makes the repair proficiency of the cell or clone of paramount importance in generating the end points.

From the dose response curves for low LET it seems that all Tradescantia strains at a dose rate above about 0.3 Gy/min have increasing probability that compound coincident lesions lead to end-points. Even at high dose rates, cells need to have more than 5 - 10 cGy to begin to show the dose rate effect. So to that extent there is a curve inflection (not a threshold), changing from a slope of +1.0 to a greater slope generally around +1.4.

The deduction is that low LET lesions can never reach the levels of coincidence in time and space observed for high LET radiations. Thus the probability of lesions in the target leading to a genetic event will never be the same for the two types of radiation. Because repair is involved in generating the end-points, the further deduction is that repair systems will saturate long before the probability of low LET radiation induction of end-points reaches unity with high LET induction.

Dwarf cells were also scored in the Clone 02 data. These can be blue, pink, or colorless, but they arise independently of the "pink" color change. They arise with a slope of ~+1.4 of high LET radiation, in contrast to all other end-points of high LET, which arise at +1.0 or less. At the same time, they are also induced with a response curve of +1.4 with low LET radiation, marking one of the few data sets from Clone 02 in which the low and high LET data have the same yield slope.

Both high and low LET yield curves for "dwarf" cells originate at much higher relative doses than any of the other end-points scored for either radiation. In other words their yields depend upon the presence of many more hits than the other end-points. But at the same time, their yields never intercept the absolute yields observed of high LET "pink" events. The interpretation is that "dwarf" cells always result from more than one hit, in more than one target, but that their absolute yields are limited by cell death, and that the probability for each of the independent events smoothly increases with dose.

For high LET, "normal" goes to "dwarf" at a 10-fold lower frequency than to "pink" in Clone 02, with a slope of ~+1.4; "normal" goes to both "colorless" and "dwarf" with a yet lower frequency and a slope of ~+1.4. And "normal" goes to "pink" and "dwarf" about 10 fold less frequently than either alone, with a slope of ~+1.0.

Assume that the genetic changes to "pink", "colorless" and "dwarf" are independent results of hits in different targets. For example, "dwarf" may represent a deletion of part of a chromosome which has cell size genes on it; "colorless" may reflect deletion of both hair color genes and their relevant chromosome arms, and "pink" may reflect mitotic crossing-over between the color genes and the centromere.

Then mitotic crossing-over of "blue" to "pink" proceeds with 100% probability for high LET hits in the target. "Blue" goes to "colorless" with an efficiency of less than 100%, possibly because some deletions lead to death. "Blue" goes to "dwarf" with an initial

yield less than 10% of the yield of e.g. "blue" to "pink", (implying that the target is 1/10 as big) but as there is a slope of ~+1.4, high LET lesions must interact to increase the probability of a deletion.

The double deletion "blue" to "colorless" "dwarf" has a yet lower efficiency, but the same slope of about +1.4. The increase in yield with increasing dose implies that the probability of two deletions in the same cell after more than one ionizing radiation event goes up. This may be expected if deletions result from incomplete repair, and single high LET tracks come near to saturating repair processes. This suggestion is supported by the generally exponential high LET lethality curves for Tradescantia.

Finally, the combination of a "pink" mitotic cross-over, and a "dwarf" deletion has a probability less than 10% that of the "pink" event alone, and a slope of +1.0. This suggests that the repair events which generate deletions and "pink" events are independent, and that there is no interaction between the deletion events in cells which also give rise to a mitotic cross-over.

So the interpretation is that there are further repair processes than just recombination acting after high LET radiation, and that the interaction of repair processes which result in deletions leads to a higher probability of double deletion than of single deletion, or of deletion with or without mitotic crossing over.

An alternative physical interpretation is that deletion represents the running together of two repair events, starting from nearby lesions, and resulting in a "repair collision" which cannot be resolved without deletion. In this case, some coincident lesions would be very likely to lead to deletion, and others not.

Poisson distribution of lesions in large targets and the probability of LOH

The dose rate data (1 and 4) suggests that at dose rates around 0.04 cGy/min of low LET radiation there is a significantly lower probability for a lesion to lead to a mitotic cross-over than at a dose rate between 0.3 and 4 Gy/min. Two hypotheses are explored to explain this observation.

In the first case, it could be imagined that the coincidence of two lesions in the same target, or in the target plus the attached chromosome arm, at the same time, would increase the probability of mitotic crossing-over. This probability should, therefore, increase as the Poissonian probability of coincidence of DSB and/or LCD in the target and/or chromosome arm.

An alternative hypothesis is that repair is rate limited by the amount of a hypothetical "repair complex" present in the cell. At low doses and dose rates repair can proceed with a low probability of "S" resolution of recombinational junctions, but as the number of junctions present in a cell goes up, either as a function of dose or dose rate or both, junction resolution increasingly often is of the "S" type. This could happen if, for

example, some part of the complex which catalyzed "R" resolution, such as the enzymes which govern the degree of superhelicity of the DNA, was rate limiting.

Table A6-1 gives the Poissonian expectations for lesions in Clone 02 cells irradiated with low LET radiation. In a target of 6×10^{10} base pairs 1 cGy is expected to induce 2.7 DSB and 1.2 LCD per cell. In 10,000 cells, expect the following distribution of numbers of lesions per cell, based on a mean of 3.9 lesions per cell, and the derived number of lesions in the same chromosomal target of 2.6×10^{8} base pairs (1/233 of the total).

From Reference 1 the expectation is for 6 "pink" events to arise, at the low LET dose rates used in those studies. At the lowest dose rates this reduces to 3 expected, for an efficiency of 3/167 or 1.8%. But at the dose rate used, 1 lesion in 25 of low LET, in the target, leads to mitotic crossing over. At the highest dose rates 16 lesions in 167 in the target, or 10%, lead to the mitotic crossing-over end-point. These calculations do not prove that more than one hit needs to occur in the same target, or in the target plus the adjacent chromosome arm. However the observed probability of chromosome arms with more than one hit in them is consistent with the argument that recombination probability goes up as a function of compound coincident lesions.

Table A6-1: Clone 02 cells with hits, and the frequency of single hits in the chromosomal target containing the "pink" gene, based on 10,000 cells at risk and 3.9 coincident lesions per cell at 1 cGy of gamma irradiation. Also the number of lesions expected in the rest of the chromosome arm, and the frequency of coincident lesions in both the target, and the rest of the chromosome arm.

# Lesions:	# Cells:	# Lesions/Target:	Lesions/Chrom.	Coincident Lesions:
0	202	0	0	0
1	789	3.4	31	0.1
2	1539	13.2	119	1.0
3	2001	25.8	232	3.0
4	1951	33.4	301	5.2
5	1522	32.7	294	6.3
6	989	25.5	230	5.9
7	551	16.6	149	4.5
8	269	9.2	83	2.8
9	116	4.5	41	1.6
10	45	1.9	17	0.7
11	16	0.8	7	0.4
12	5	0.3	3	
Totals:	10,012	167.3	1507	31.5

Calculation of coincident hits in both the target, and in the rest of the chromosome arm suggests that there may be a direct correlation between coincident lesions in the target and in the rest of the chromosome arm for mitotic-crossing-over to occur.

There are expected to be 32 chromosomes with a hit in the target and in the rest of the chromosome. At the highest doses and dose rates, there are about 16 "pink" events, and at the lowest about three. So on a coincidence hypothesis, there would be a 50% probability of generating a "pink" in chromosomes with a hit in the target and the arm, versus a 10% chance for a hit in the target alone. And at the lowest doses and dose rates, there would be about a 10% chance for hits in the target and the chromosome arm, versus about a 2% chance for a hit in the target alone.

For high LET radiation the Poissonian expectations for the numbers of lesions per cell is given in Table A6-2 column 1; column 2 gives the numbers of cells out of 10,000 with the given numbers of hits; and column 3 gives the numbers of targets expected to have a lesion in them. As argued above, the expectation is that high LET lesions will always include coincident DSB and LCD, certainly within the size of the defined target.

At 1 cGy of 0.43 MeV neutrons, the observed production of "pink" hairs is 3/100 or 300/10,000 which is 77% of the total calculated number of hits in the target. From Figure 6, reference 1, the expectation of a "pink" event at 0.01 cGy of 0.43 MeV neutrons is 3 per 10,000 cells. In Column 3 the expected numbers of lesions in the target coincides exactly (by radiation biological standards!) with the observed numbers of "pink" events at these doses. The generation of end-points is linear regardless of dose rate.

So for high LET the hypothesis of obligatory coincident lesions is in agreement with the facts, and of a high probability of "S" resolution of repair junctions. The coincidence between the numbers of hits in the target, and the numbers of "pink" events observed is unlikely to be random.

From the data on the effects of dose rate on survival of both Clone 02 and T. blossfeldiana, tabulated in A6-1, it is clear that lesions coincident in time are more lethal than those in which the dose takes longer. So the time component of repair can be hypothesized to reflect the need for a higher amount of "damage repair complex" to deal with all the damage simultaneously. In other words, the requirement for DNA repair enzymes is absolutely less if the dose is protracted, because one repair event can be completed before the next is started.

The interaction kinetics observed at high doses and dose rates of low LET radiation (a slope of greater than +1.0) reflects a steadily increasing probability that a repair event will lead to the genetic end-point. If this increase is because more of the cells' repair capacity is being used at the same time, the implication is that the mopping up of repair complexes at lesions, as such, leads to a higher probability of segregational resolution of repair structures.

Table A6-2 Numbers of lesions in the "pink" target after 1cGy of 0.43 MeV neutron irradiation on the assumption of 9.1 lesions per cell in 10,000 cells and a target of 2.6 x 10^8 base pairs:

# Lesions:	# Cells:	# Lesions/Target:
0	1	0.0
1	10	0.0
2	46	0.4
3	140	1.8
4	319	5.4
5	581	12.5
6	881	22.7
7	1145	34.4
8	1302	44.7
9	1317	50.9
10	1198	51.4
11	991	46.8
12	752	38.7
13	526	29.3
14	342	20.5
15	208	13.4
16	118	8.1
17	63	4.6
18	32	2.5
19	15	1.2
20	7	0.6
Totals:	10,000	389.9

Repair complex saturation is consistent with the experiments done at reduced temperatures, where the dose rate effect is decreased (A6-2). If, at the low temperature, repair is largely blocked, then on incubation at the normal temperature the numbers of lesions available to "mop" up the repair complexes will be greater and a psuedo-dose rate effect will be observed, as is the case. The repair rate at the lower temperatures will be affected by the usual drop of c.a. two-fold for each 10°C drop in incubation temperature.

Mericle and Mericle (A6-6) have measured micronuclei associated with sectors and observe that they account for between 1/200 and 1/300 of the nuclear volume and hence DNA. So if the loss of 1/200 of the nuclear DNA can give rise to a deletion sector, the recombination of the same amount of DNA can give rise to mitotic crossing-over. This defines the "pink" target.

Finally, three lesions in 163 of low dose low LET lead to "pink" and 300 out of 389 of high LET, within the same target. The overall efficiency, taking both increased lesion induction, and more efficient LOH induction, for high LET is thus about 40-fold. As is calculated in Appendix 7, this is again in good agreement with the 22-fold greater efficiency observed when comparing intermediate dose rate low LET with high LET radiation.

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APPENDIX 7

LESIONS/CELL, LOH EVENTS PER CELL, AND LESIONS/LOH EVENTS PER TARGET AND PER CELL

In Appendix 6, Poison calculations were performed to obtain the numbers of lesions in the Tradescantia Clone 02 "pink" target, to see if multiple hits in the same repair domain lead to a higher probability of "S" events per lesion. Using the same yield assumptions, it is also possible to compare the absolute numbers of lesion in a cell with the expectation of a "pink" event. This graphic method has the advantage that it aids in the interpretation of dose response curves.

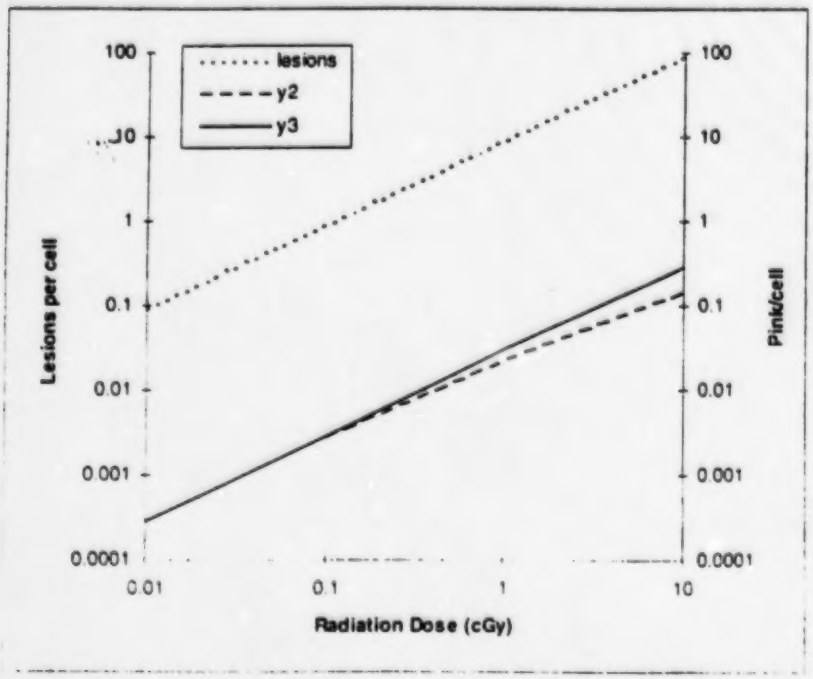

Figure A7-1: The numbers of lesions per cell induced by 0.43 MeV neutrons in Tradescantia Clone 02 in comparison with T. subacaulis plotted on a log-log plot.

The yields of DSB and LCD (from Appendix 2) for high LET radiation is plotted on the assumption of linear induction of DNA damage with increasing dose. Also plotted on the graph is the frequency of "pink" events per hair in Clone 02, line y3, and for comparison from T. subacaulis , line y2 . Because of the division pattern of hair development, one "pink" event per hair equals one "pink" event per cell in the initial hair precursor population.

For Clone 02 the slopes of lesions and yields are parallel and one lesion in about 300 per cell gives rise to one "pink" hair. For T. subacaulis, the yield line intersects with the Clone 02 line at low doses. This means that at the lowest doses, there is close to a unit probability of inducing a "pink" in either Clone 02 or in T. subacaulis by a high LET hit in the target.

The slope of +0.85 for "pink" induction in T. subacaulis indicates that the probability of a "pink" does not go up linearly with dose. In fact, the probability decreases smoothly with increasing dose.

For T. subacaulis, the repair of lesions to give rise to "pink" must decrease smoothly with dose. If a constantly increasing proportion of lesions is repaired by a non-productive pathway, such a dose response curve can be explained. But for Clone 02 the proportion of damage which is repaired productively must remain constant.

If Clone 02 is partially defective in LCD repair, more lesions are repaired by recombination. Because the response remains linear with dose, it appears that the probability of a "pink" event is constant even at relatively high doses of 0.43 MeV neutrons.

The probability of a "pink" event for Clone 02 is about 1 "pink" for 300 lesions in a cell. If the lesions are clustered, as suggested by ACRP-18, and if more than one lesion needs to coincide for an LOH event, then the target is 1/300 of the genome of Clone 02, or 2.6 x 10^8 base pairs. In this target, one high LET ionization leads with high probability to a "pink" somatic genetic change.

However, the probability of a "pink" in T. subacaulis drops smoothly as the dose increases. Because the production of "pink" events per lesion is becoming smoothly less probable, at 10 cGy only 1 lesion in about 600 leads to the event in this wild-type strain. The probability of a lesion being in the target does not decrease with increasing dose even while the probability of a "pink" is decreasing. So one concludes that the distribution of lesions to productive and non-productive repair systems is changing the probabilities of LOH in wild-type T. subacaulis.

The expectation of decreasing yield with increasing dose of high LET radiation would also hold for wild-type human or other mammalian species. A decreasing yield of LOH would be expected if the amount of recombinational repair enzymes remains constant, or increases at a lesser rate than for LCD repair enzymes.

In Figure A7-2, the low LET yield of lesions per cell and of "pink" events per cell are plotted on a log-log scale. From the RBEs in Appendix 2, Table A2-3, the relative numbers of DSB and LCD per cell can be computed as a function of dose. Then, plotting the yields of "pink" events per cell allows the same calculation as for the yields after high LET radiation.

Corrected for lower lesion cross-sections, and plotting as the solid line the linear part of the low LET "pink" curve from Reference 1, the production of "pink" events per lesion can be calculated. It turns out that about 1 lesion in 8,000 in a cell of Clone 02 leads to a "pink" event after low LET radiation at low dose rates. If the target is the same size as for high LET radiation, this means that low LET radiation is about 1/26 as efficient at generating LOH events per lesion in the target in Clone 02 as high LET radiation.

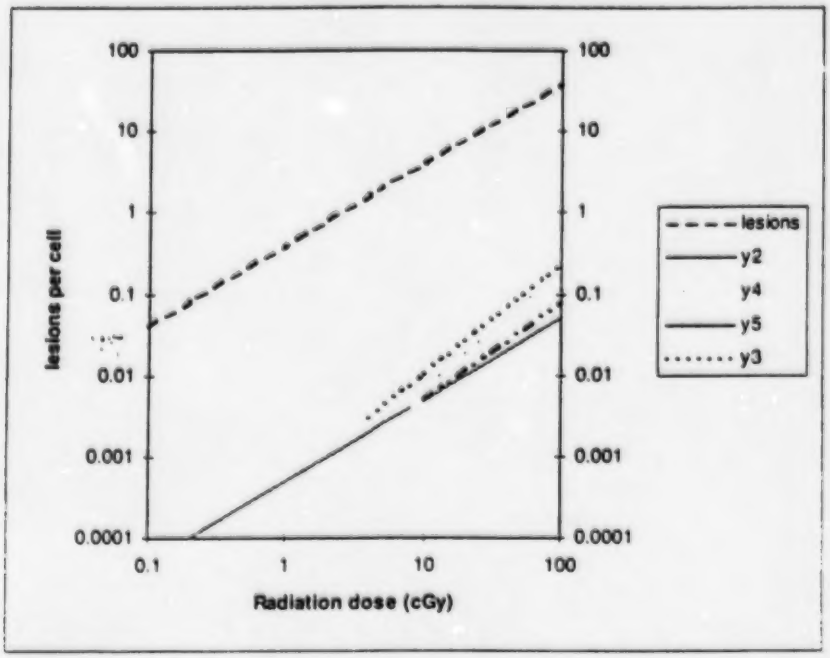

Figure A7-2: Line y2 is the low dose rate yield for Clone 02; line y4 is the yield in Clone 02 at 0.3 cGy/ min; line y3 is the yield in Clone 0106, and line y5 is the yield in T. subacaulis. 150 kVp X-rays at <0.5 mGy/min to 0.3 Gy/min.

At high dose rates, all three strains yield more "pink" events per lesion. For high dose rates, by 1 Gy at 0.3 Gy/min, the probability is that 1 lesion in 1600 leads to "pink" in Clone 0106; 1/2000 in Clone 02, and about 1/3500 in T. subacaulis. Again, assuming the target does not change in size, the number of lesions coinciding in the target spatially and temporally can explain this observation.

For Clone 02, 1 lesion in ~20 in the target at the lowest doses and dose rates leads to "pink". There is no data from wild-type Tradescantia or other species for LOH yields at low doses and dose rates of low LET radiation. But the data from yeast and lower eucaryotes suggests that the yield of LOH is linear to the lowest doses and dose rates.

None of the data suggests that low LET lesions at any dose or dose rate will ever be as effective at inducing LOH in Tradescantia as high LET lesions. Thus, for high doses and dose rates of low LET radiation, in Clone 0106 ~ 1 lesion in 5 in the target leads to LOH (compared to the high LET efficiency of 1.0); in Clone 02 about 1 in 7, and in T. subacaulis, about 1 in 12. If the targets are the same size, then variations in repair efficiency, lesion channeling and lesion clustering explain the ultimate, absolute differences between low and high LET lesions and their repair.

Table A7-1 tabulates the data. In summary, the Table shows the effects of dose rate, LET, and Tradescantia strain on the generation of the "pink" end-point. Of course, in addition to dose rate of low LET there is the further effect of the absolute dose of low LET at a particular dose rate. It turns out that dose responses to low LET radiation are affected by a large number of variables which do not affect responses to high LET radiation.

Table A7-1: Variation in efficiency of inducing "pink" events per lesion of high or low LET radiation in different Tradescantia clones and species.

Clone:	LET	Dose rate	Pink/lesion:
02	0.43	Instantaneous	1/300
02	250 - X	0.4 cGy/min	1/8000
02	250 - X	0.3 Gy/min	1/8000 - 1/2000
0106	250 - X	0.3 Gy/min	1/1600
T. subacaulis	0.43 MeVn	Instantaneous	1/300 - 1/600
T. subacaulis	250 - X	0.3 Gy/min	1/3500

The data suggests that Clone 02 is the simplest situation in the plant LOH studies. With different strains, the LOH functions associated with radiations of differing LET, dose, and dose rates vary as a further component of repair proficiency.

It should be noted that in studies of the linked induction of micronuclei versus other cellular changes, the Mericles point out (A7-1, A7-2) that the average volume of a radiation-induced micronucleus is about 1/300 of the nuclear volume. This is remarkably similar to the radiation target defined in the above studies. But the Mericles have also obtained evidence that the generation of "pink" events is negatively correlated with the induction of micronuclei, so "pink" events cannot simply be the result of single chromosome arm deletions.

Further calculations about lesion yields per target were carried out to examine the dose response curves expected if two hits need to occur in the target to generate the end-point. A combination of lesion yields and Poissonian probabilities are used to calculate the numbers of "pink" targets expected at different doses with one or more coincident lesions in them, and then compared with the observed number of "pinks" generated.

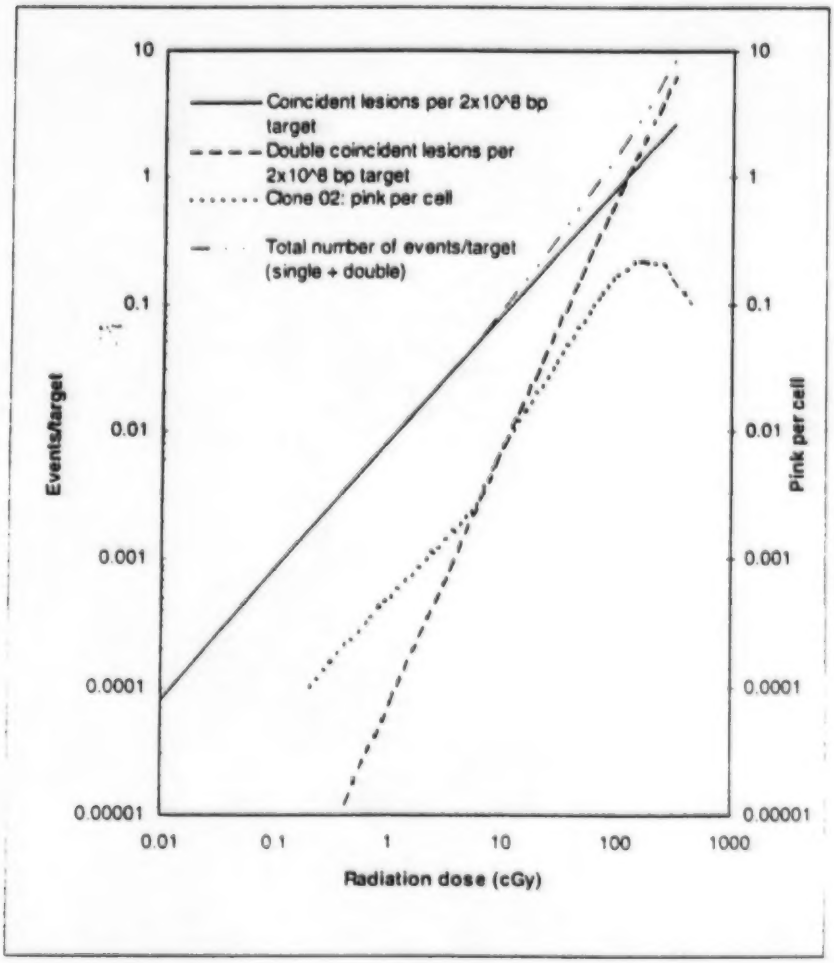

Figure A7-3: The numbers of single and double lesions expected in the Tradescantia target (defined by the high LET yields) as a function of low LET dose, and the observed yields of "pink" events from hits in the target.

Using the DSB and LCD yields detailed in Appendix 2 and the "pink" target size derived from the 0.43 MeV neutron yield data, it is possible to test the hypothesis that two low LET lesions need to coincide in the "pink" target to generate a "pink". The D^2 curve of

lesions in the target is for all combinations of DSB and LCD at a ratio of roughly 2 DSB : 1 LCD.

The summed single and double hits in the target give the probability of one or more hits in the "pink" target. The hit probability smoothly increases from [illegible] at low doses to 2 at higher doses. At slopes of +2 hit probabilities, the "pink" yield curve saturates, and the survival curves given in Figure 1 of the main text indicates that cells are being killed. So at multiple hits in the target the probability of "pink" events goes down, and the probability of death goes up. This fits with models of repair saturation.

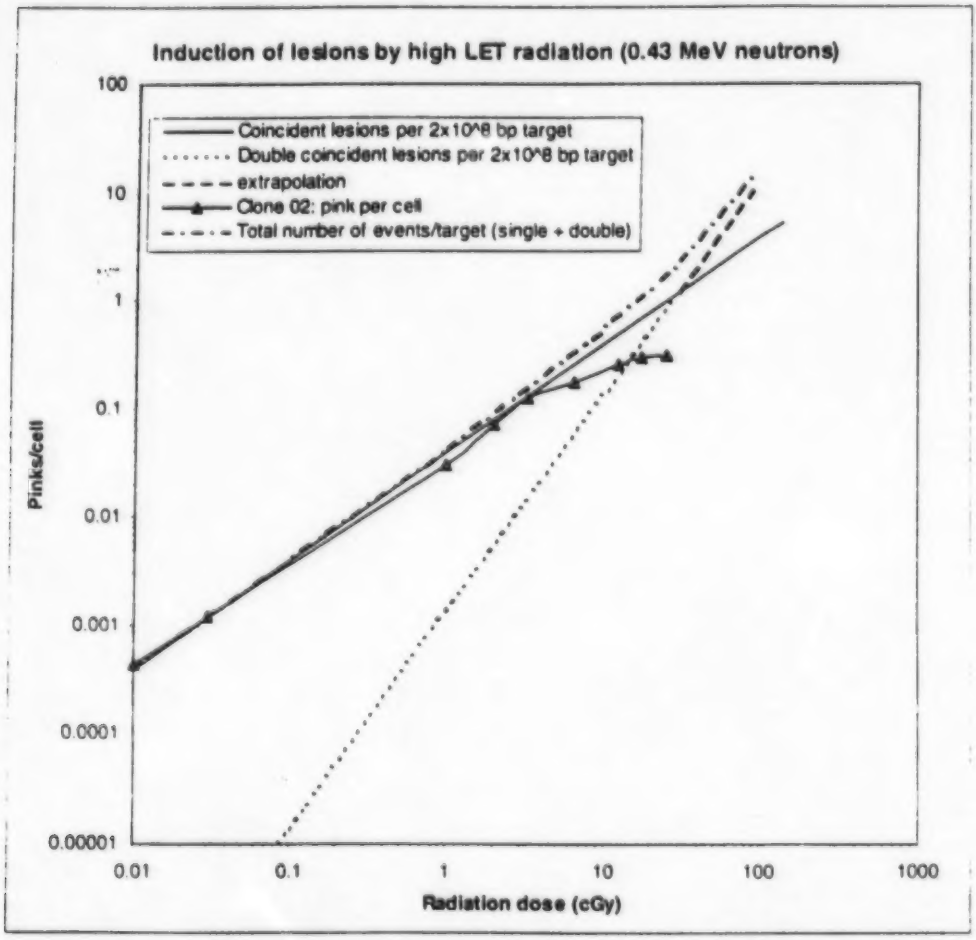

Figure A7-4: The yield of "pink" events on 0.43 MeV neutron irradiation, relative to single and double hits in the target.

The data in Figure A7-3 rule out the necessity for two hits to occur in the target to yield one "pink" event. From the double hit curve, the coincidence of double and single hits in the targets at more than 6 cGy leads to the production of more "pink" sectors per cGy.

The solid line gives the number of hits in the target, and the yield of "pink" hairs is given by the hatched line. The number of double hits in the target is given by the line with the slope of +2.0. Finally, the number of targets with two or more hits is given by the smoothly curving line of joint probability. Generation of "pink" hairs per lesion is near 80% per lesion in the target. This should be compared with the ~1/20 probability that a low LET lesion in the target will lead to a "pink" hair, rising to about 1/5 before the yield curve saturates as lethality becomes detectable.

In this data there is no indication that two hits are more effective than one, or that increasing doses result in anything other than repair saturation and hair precursor death. If the assumptions are truly based, there is no question but that the efficiency of generating the end-points is much higher for high LET lesions than for low.

Figure 7 in the main text combines the data given in Figures A7-3 and A7-4 to summarize the data.

At 1 Gy of low LET radiation there is an expectation of about 1.7 lesions per target. The Poissonian probability is that about 20% of cells will have 0; 30% will have one hit in the target, and the remaining 50% have two or more. From the low dose part of the "pink" curve, about 1of 16 lesions in the target will lead to a "pink" when the probability of a second lesion is negligible.

At 1 Gy, the number of "pinks" expected is about 0.2/cell at risk. If the same proportion come from single lesions, which make up 30% of hits in targets, then 1 lesion in 16 of the single lesions leads to a "pink" to generate 0.03 "pinks'. If the remaining 0.17 events occur in the 0.85 cells with two or more lesions, then about one lesion in five of double lesions in the target leads to the "pink" recombination event.

So the probability of double lesions in the same target leading to the "pink" event is greater than for single lesions by about three-fold. But for low dose, low dose rate, low LET radiation the figure of 1 lesion in 16 (or 1 in 100 for wild-type strains) can be used to calculate the probability of LOH. This would correspond to the typical low dose, low dose rate, low Let exposure.

For low LET radiation, the yield of "pinks" is parallel to the slope of lesions per target until about 5 cGy. This coincides with the point at which targets with more than one lesion start to form a significant fraction. The double lesions go up at a significantly faster rate than do the "pinks at a dose-rate of about 0.3 Gy/min, at which dose rate effects are minimized. At 0.4 cGy/min (A7-3), the slope of "pinks" versus dose is only +1.05, at doses up to 1.5 Gy, at which each target would have significantly more than one hit (nine hits, according to Figure A7-3).

Thus, while low doses of low LET have some unit probability of generating a "pink", higher doses and dose rates do not have a dose-squared relationship. None of the data from Tradescantia allows a dose response curve of more than +1.7, and most are +1.4 or less. Explanations for this deviation from the simplest explanation must have to do with interactions between DNA repair events, some of which lead to the end-point and some of which do not.

The other factor to consider is that in all the Tradescantia studies doses above 1 to 3 Gy show a saturation phenomenon in which no more "pinks" are induced, and at which cell survival declines to the LD_{37}. At the LD_{37} one would expect about 3 - 4.5 coincident lesions per 2.6×10^8 base pair repair domain. That is, each "pink" equivalent target in the cell would have multiple hits.

Death by Hitting the Repair Genes

The saturation phenomenon, related as it is both to cell death, and cessation of end-point generation, is most easily understood as reflecting saturation of the recombinational repair systems which repair DSB. If about 1% of the genome is taken up by DNA repair genes, one would expect to be inactivating DNA repair genes at the LD_{37} leading to the ultimate death of the cell.

There would be about 200 genes per repair domain, including two repair genes; about three hits per repair domain, and a reasonable chance that one of the repair genes was hit. In fact, of the approximately 600 repair genes, at 1 Gy one would expect to have hit two of them at least 50% of the time. So the hypothesis that cell death results from hits in the structural genes for DNA repair functions is probabilistically plausible. Note that despite the fact that Tradescantia has 10 times as much DNA as humans, no correction has been made for the numbers of genes expected, nor for their size.

If the same assumptions are made for T. blossfeldiana then a significantly higher number of hits is required to inactivate either cells or the genes vital in cell survival. This is again explicable on the grounds that wild-type strains have the possibility of recycling repair systems until viable genomes are reconstituted, and this will depend on the correct functioning and interrelationships of the various repair systems.

The suggestion that cell death reflects hits in DNA repair genes was first made by Holliday (A7-4) in the original report of the induction of DNA repair systems in the smut fungus Ustilago maydis. It is interesting that calculations in Clone 02 suggest that this is a reasonable expectation. In Table A7-2 the LD_{37} for the various yeast mutants are compared in terms of the probability of a hit in one of the 6% of genes involved in replication, recombination and repair (RRR) functions. Also included for interest is a calculation of the number of hits required in Ustilago wild-type, which has an LD_{37} of about 4,500 Gy, and a genome twice the size of yeast.

The data indicate that while coincident lesions in yeast RRR genes may lead to lethality, for repair defective strains they are neither necessary nor sufficient to account for cell death. Only in wild-type may they be both necessary and sufficient to lead to cell death. So in this instance, inactivation of RRR genes may actually lead to cell death.
For Ustilago, several factors militate against a correlation between hitting RRR genes and cell death. It has twice the genome size of yeast, but has a largely G2 cell cycle. This means that sister-chromatid recombination repair is always available. As a parasite, it has a life style of meiotic hyper-recombination to produce new meiotic variants which can attack its host plants. For these recombination repair related reasons, then, 39 lesions in the same RRR genes are needed for one average lethal hit. So in this example, the importance of DNA repair is obvious from the enhanced ability it confers to survive lethal lesions.

Table A7-2 : LD_{37} and Poissonian distribution of coincident lesions in RRR genes of yeast and smut:

Strain:	RRR Defect:	LD_{37}	Number of RRR genes @ 1+ hits:	
Wild-type	None	260 Gy	67% +1 hit	+N+S
RAD6	LCD	115 Gy	40% + 1 hit.	-N-S
RAD 52	DSB	65 Gy	25% + 1 hit	-N-S
RAD 51	DSB	20 Gy	10% +1 hit	-N-S
U. maydis.	None	4500 Gy	39 hits	-N-S

Finally, in T. blossfeldiana assuming the same target size as Clone 02, between 3.5- and 4.5-fold more lesions are required for a lethal hit as in Clone 02, another indication of the importance of DNA repair both in end-point generation, and in cell survival.

So the complications of interpretation of cell killing and survival arise from both the Poissonian expectation of multiple hits in critical targets, and repair competence which varies between cell strains and species as a function of the capacity for DNA repair of the strain and organism.

In fact, what is expected is that there will be a linear induction of lesions in the target until the doses are high enough for coincident lesions to be induced. Therefore, the induction of genetic events as a function of dose is expected to have a slope of one until enough coincident lesions occur to change the induction slope. However, for reasons

having to do with the molecular mechanisms of lesion repair and its resolution, a dose-squared relationship is not observed in the Tradescantia data.

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